EASTERN VIRGINIA MEDICAL SCHOOL
OFFICE OF RESEARCH
[redacted]
NORFOLK, VIRGINIA 23507-2000

EASTERN VIRGINIA MEDICAL SCHOOL

TELEPHONE [redacted]
FAX [redacted]

July 13, 2009

Gerald J. Pepe, Ph.D.
Dean and Provost
Department of Physiological Sciences
Eastern Virginia Medical School
[redacted]
Norfolk, Virginia 23507

Dear Dr. Pepe: Jerry

Your response to the June 12, 2009 letter regarding the protocol entitled, ***Regulation of Fetal-Placental Development in the Primate*** **(IACUC #09-007)**, has been reviewed and accepted by the Institutional Animal Care and Use Committee. **The project is now approved for one year.** Continued approval beyond this point will require submission of an annual progress report, no later than **June, 2010**.

It is the responsibility of the principal investigator to notify the Committee, in writing, of any deviation from the IACUC-approved protocol. Please note that a change in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian prior to submitting the amendment request to the IACUC for approval.

It is also the responsibility of the principal investigator to provide for regular observation of the health of the research animals and to provide the Division of Comparative Medicine (CompMed) with the name(s) and telephone number(s) of the person(s) who may be contacted and/or take action in the event that the CompMed staff notes an emergency condition with the research animals.

Thank you for your continued cooperation with the Institutional Animal Care and Use Committee.

Sincerely,

[redacted]

[redacted], *Chair*
Institutional Animal Care and Use Committee

[redacted]

cc: [redacted]
Interim Department Chair

[redacted]
Interim Attending/Clinical Veterinarian
Division of Comparative Medicine

[redacted]
Interim Program Manager
Division of Comparative Medicine

[redacted]
Associate Dean for Research
Institutional Official

EASTERN VIRGINIA MEDICAL SCHOOL
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

RECEIVED
JUN 25 2009
OFFICE OF RESEARCH
EVMS

FOR IACUC USE ONLY:	
IACUC Number: 09-007	Review Date(s):
NOTES: Revision #1 FINAL	Final Approval Date: 7/15/09
	Progress Report Due: 6/10/10, 6/10/11

Submission Instructions: Submit the signed original typed form, along with **seventeen (17)** photocopies to the IACUC Office located in [redacted] no later than 5:00pm on the submission deadline date. Forms received after the deadline will be held until the next IACUC meeting. For assistance, please contact the IACUC Administrator at [redacted]

Initial Review Form for New Animal Care and Use Protocols

Project Title: *(If project title is different from the grant title, please list both titles below)*
Regulation of Fetal-Placental Development in the Primate

Is this a 3-year renewal of an existing IACUC protocol?	☐ NO	☒ YES	Related IACUC #:	06-005

SPECIES INFORMATION: (*In addition to the species, please list the strain(s), if applicable, the sex(es,) and the age(s) of the animals.*)
Baboon (Papio anubis/cynocephalus)

Principal Investigator:	Gerald J. Pepe, Ph.D.		
Mailing Address:	Department of Physiological Sciences Eastern Virginia Medical School		
Phone: Office:	757-[redacted]	Home:	[redacted]
Lab:	757-[redacted]	E-mail:	[redacted]

Animal Emergency Contact Person:	[redacted]		
Phone: Office:	757-[redacted]	Home:	757-[redacted]
Lab:		E-mail:	[redacted]

Technical Coordinator:			
Phone: Office:		Home:	
Lab:		E-mail:	

Co-Investigator #1:	[redacted]		
Phone: Office:	757-[redacted]	Home:	
Lab:	757-[redacted]	E-mail:	[redacted]

Co-Investigator #2:			
Phone: Office:		Home:	
Lab:		E-mail:	

Co-Investigator #3:			
Phone: Office:		Home:	
Lab:		E-mail:	

Co-Investigator #4:			
Phone: Office:		Home:	
Lab:		E-mail:	

LIST ALL PROJECT SITES:				PROJECT PERIOD:		
Bldg:	[redacted]	Rooms:	[redacted]	From: June 1, 2009		To: June 1, 2012
Bldg:		Rooms:				

FUNDING SOURCE(S):	***Please check all that apply.*** ☒ Federal Government ☐ State or Other Government ***Specify Source:*** NIH ☐ Private ☐ Industry ☐ Campus/Department Funds ☐ Other ***Specify source:*** _____		
STATUS OF FUNDING:	☒ Approved HD 13294	☒ Pending - R01 Supplement to U54 HD 36207	☐ Not Applicable
Is a committee approval verification letter needed for the funding source(s)?	☒ NO Please note that it is the investigator's responsibility to inform the funding agency of any changes to the animal protocol. Any changes must also be approved by the IACUC before they are implemented.	☐ YES (***Complete Attachment A, REQUEST FOR A LETTER OF VERIFICATION***) ☐ Final copy of grant attached Please include a final copy of the grant to permit comparison of the animal work described in the grant with the animal work described in the *Initial Review Form*.	

OTHER COMMITTEE REVIEWS:

Prior to the initiation of this project, approval must be acquired from the appropriate committees or offices.

Please complete the following table as it pertains to your protocol. If applicable, complete ***Attachment D, USE OF HAZARDOUS AGENTS:***

Project Involves:	Yes	No	Committee/Office	Certification Number	Hazard to:	
					Personnel	Animals
Radioisotopes, *in vivo*	X		EVMS Radiation Safety Committee *(Complete **Attachment D**)*	A-022 Submitted	X	
Recombinant DNA, RNA, Primate Tissue Culture, Laboratory Induced Infection, Cultured Pathogens	X		EVMS Institutional Biosafety Committee *(Complete **Attachment D**)*	01-017 Submitted for review by IBC on 7/13/09; will update once approval is granted	X	
Known or Suspected Chemical Hazards, Mutagens or Teratogens		X	EVMS Office of Environmental Health & Safety/Radiation Safety *(Complete **Attachment D**)*			
Lasers or Penetrating Electromagnetic Radiation with Living Animals		X	EVMS Office of Environmental Health & Safety/Radiation Safety			
Other (*Please describe*): X-ray	X				X***	

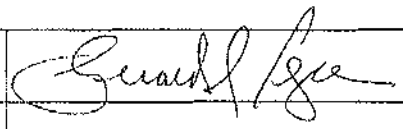
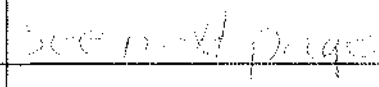
***Studies performed by [redacted] and [redacted] in collaboration with Comparative Medicine

PRINCIPAL INVESTIGATOR'S ASSURANCES:

I hereby certify that:

- no animal involved in this project will be subjected to discomfort, pain, or distress unless it is unavoidable in the conduct of scientifically valuable research;
- any such discomfort, pain, or distress will be alleviated with the appropriate anesthetic, analgesic, or tranquilizing drugs unless specific approval is given by the Committee for not using these agents;
- the project will be carried out within the provisions of the Animal Welfare Act (Public Law 99-198), the National Research Council (NRC), Public Health Service Policy on Humane Care and Use of Laboratory Animals (PHS), the "Guide for the Care and Use of Laboratory Animals (1996)", the Health Research Extension Act of 1985 Public Law 99-158 (11/20/86), and United States Department of Agriculture (USDA) regulations;
- all procedures in this protocol and/or personnel changes will be brought to the attention of the IACUC through the amendment process, prior to implementation, and that failure to request an amendment for changes in animal use may place the School and myself in violation of Federal regulations and the Animal Welfare Act;
- the details of the research to be conducted in this protocol are consistent with the details of the research as written in any grant, contract or subcontract related to or connected with this protocol;
- all personnel using animals have completed the appropriate training requirements to assure the humane, safe and appropriate use of animals in this context.

The signatures enlisted below signify assurance that the individuals involved will comply with the project as described herein.

Principal Investigator:		Date:	5/6/09
Technical Coordinator:		Date:	
Co-Investigator #1:		Date:	
Co-Investigator #2:		Date:	
Co-Investigator #3:		Date:	
Co-Investigator #4:		Date:	

PRINCIPAL INVESTIGATOR'S ASSURANCES:

I hereby certify that:

- no animal involved in this project will be subjected to discomfort, pain, or distress unless it is unavoidable in the conduct of scientifically valuable research;
- any such discomfort, pain, or distress will be alleviated with the appropriate anesthetic, analgesic, or tranquilizing drugs unless specific approval is given by the Committee for not using these agents;
- the project will be carried out within the provisions of the Animal Welfare Act (Public Law 99-198), the National Research Council (NRC), Public Health Service Policy on Humane Care and Use of Laboratory Animals (PHS), the "Guide for the Care and Use of Laboratory Animals (1996)", the Health Research Extension Act of 1985 Public Law 99-158 (11/20/86), and United States Department of Agriculture (USDA) regulations;
- all procedures in this protocol and/or personnel changes will be brought to the attention of the IACUC through the amendment process, prior to implementation, and that failure to request an amendment for changes in animal use may place the School and myself in violation of Federal regulations and the Animal Welfare Act;
- the details of the research to be conducted in this protocol are consistent with the details of the research as written in any grant, contract or subcontract related to or connected with this protocol;
- all personnel using animals have completed the appropriate training requirements to assure the humane, safe and appropriate use of animals in this context.

The signatures enlisted below signify assurance that the individuals involved will comply with the project as described herein.

Principal Investigator:	[redacted]	Date:	5/6/09
Technical Coordinator:		Date:	
Co-Investigator #1:		Date:	7/13/09
Co-Investigator #2:		Date:	
Co-Investigator #3:		Date:	
Co-Investigator #4:		Date:	

DEPARTMENT CHAIR'S ENDORSEMENT:
I have reviewed the research project which is the basis for this submission. I am aware of and approve of the procedures outlined for this study. I agree to take ultimate fiscal responsibility for the payment of animal care charges.

Department Chair or Designee:	[redacted]	Date:	5-7-09
Printed or Typed Name:			

VETERINARY CONSULTATION:
The IACUC requires a **MANDATORY** consultation with the veterinarian to provide the investigator with information that is relevant to the species and study procedures. It is the responsibility of the investigator to incorporate the appropriate information into the protocol **BEFORE** it is submitted to the IACUC for review. The veterinarian's signature below **does not** constitute an approval of the protocol. The signature merely acknowledges that a consultation with the veterinarian has occurred.

EVMS Veterinarian	[redacted]	Date:	5/8/09
Printed or Typed Name:			

IACUC APPROVAL:

IACUC Chair or Designee:	[redacted]	Date:	7/20/09
Printed or Typed Name:		[redacted]	

A. PROTOCOL OBJECTIVE:

In clear, concise, non-technical, lay language *(i.e. the type of writing style used in newspapers)*, summarize the background, general hypothesis, experimental plan, and relevance of the study to the advancement of scientific knowledge and/or the benefits to human and animal health. All abbreviations must be defined. **Scientific abstracts from grant applications or journal articles are not acceptable.**

Goal: The goal of these studies is to determine the role of estrogen on growth and function of the placenta and development of a fetus capable of surviving in the perinatal/neonatal period and developing thereafter and exhibiting normal adrenal and reproductive status in adulthood. Briefly, pregnant baboons will be treated with estrogen or a specific inhibitor of estrogen synthesis alone or in combination with estrogen during discrete periods of gestation to determine impact of prematurely elevating estrogen levels as well as estrogen deprivation/ restoration on aspects of placental-fetal development. Treated/untreated animals will be delivered by cesarean section at early, mid or late gestation and the placenta and fetal tissues (e.g. adrenal gland; gonad) collected and studied for aspects of biochemical/physiologic maturation. In selected experiments, treated/untreated animals will be delivered near term and neonates reared to adulthood. Development of glucose homeostasis, bone maturation and onset of puberty as well as development of normal menstrual cycles and ovarian function as well as fertility (i.e. ability to achieve and maintain pregnancy) will be assessed. Our studies serve as a model for the human and are designed to provide basic information which will enhance our understanding of the causes of fetal growth retardation and prematurity *per se* and the role of hormones on growth and function of organ systems critical for homeostasis and reproductive function in adulthood.

Rationale for use of the baboon: The relatively high incidence of human neonatal morbidity/mortality associated with low birth weight and prematurity, the high incidence of maternal disease associated with poor placental development/vascular invasion (e.g. pre-eclampsia) as well as evidence that hormonal and environmental factors impact onset of puberty and adult reproductive function require that there be a more intensive study of the physiological and endocrine mechanisms underlying placental-fetal development. Elucidation of the latter during human pregnancy, however, has been restricted by the inability to utilize invasive experimental procedures because of ethical considerations. Thus, investigators have relied primarily on determination of hormone levels in the circulation, analyses of placental and fetal adrenal/gonadal function *in vitro*, results from abnormal pregnancies, limited endocrine manipulation at the time of elective cesarean section and correlation of pregnancy outcome with adult development. Although *in vitro* approaches utilizing isolated tissues provide valuable data, these studies alone do not permit examination of the complex functional interaction existing within the maternal-fetal-placental system. Similarly, studies of placental development in rodents have yielded new information; however, there are major differences between non-primate laboratory species and primates in placentation, the qualitative nature of hormone biosynthesis, and the endocrine interaction of the fetus and placenta. The relative inability to manipulate the endocrine axis during human pregnancy, the limitations of *in vitro* studies and inability of epidemiologic studies to test cause-effect, indicate the need for *in vivo* preparations, in which invasive experimental procedures can be employed to elucidate regulation of the primate fetal-placental unit and impact on adult development. Over the past 25 years, our laboratory has been instrumental in establishing the baboon as a nonhuman primate model for the study of the endocrinology of human pregnancy. Significant similarities in the anatomy, physiology and endocrinology of the fetal-placental unit exist in pregnant women and baboons.

B. SEARCH FOR ALTERNATIVES

In an effort to minimize pain and distress, the Animal Welfare Act (AWA) regulations require principal investigators to consider alternatives to procedures that may cause more than momentary or slight pain or distress to animals. The AWA also requires principal investigators to provide a written narrative of the methods used and sources consulted to determine the availability of alternatives, including replacements, reductions, and refinements of animal use. These alternatives should be consistent with the goals of the proposed research. *Potential alternatives that do not allow the attainment of the goals of the proposed research are not, by definition, alternatives.* The "3 Rs" are defined below:

REPLACEMENT: *An alternative that will be equally informative. Replacements include, but are not limited to, in vitro models, in silico methods, invertebrate models, and vertebrate models.*

REDUCTION: *Reducing the number of animals to the minimum required to obtain scientifically valid data and demonstrating that the proposed research does not unnecessarily duplicate previous work.* Reduction includes statistical methods to reduce animal numbers, and it addresses whether or not animals can be reused for other purposes.

REFINEMENT: *A procedure that lessens or eliminates pain or distress, thereby enhancing animal well-being.* Housing, environmental enrichment, animal identification, anesthesia, analgesia, and euthanasia procedures can be refined, in addition to activities normally thought of as procedures, such as surgeries, tissue or fluid collection, etc.

The fundamental goal of the AWA and USDA Policy 12 (June 21, 2000) is to minimize pain and distress to animals; consequently, the regulations state that any proposed animal activity or significant changes to an ongoing animal activity must include the following: (1) a rationale for involving animals, and the appropriateness of the species and the number of animals to be used; (2) a description of the procedures or methods designed to assure that discomfort and pain to animals will be limited to that which is unavoidable in the conduct of scientifically valuable research, and that analgesic, anesthetic, and tranquilizing drugs will be used where indicated and appropriate to minimize discomfort and pain to animals; (3) a written narrative description of the methods and sources used to consider alternatives to procedures that may cause more than momentary or slight pain or distress to the animals, and; (4) the written assurance that the activities do not unnecessarily duplicate previous experiments.

DATABASE SEARCHES

A database search is considered to be the most effective and efficient method for demonstrating compliance with the federal regulations for consideration of alternatives to painful and distressful procedures, although other sources, such as conferences, colloquia, subject expert consultation, etc., may provide relevant and up-to-date information regarding alternatives, in lieu of or in addition to a database search. **Institutional policy requires investigators to specify at least two (2) databases or other acceptable sources** that were used to determine that alternatives to animals have been considered, that the minimal number of animals have been requested, that the proposed research is not duplicative of previous work, and that alternatives to procedures that may cause more than momentary or slight pain or distress to the animals have been considered. For all database searches, the following information **must** be provided: (1) the name of the database; (2) the date the search was performed; (3) the time period covered by the search, and; (4) the key words and/or the search strategy used.

Please be sure to list all key words and key word combinations used and the number of citations found for each key word or combination [*e.g. amiloride mouse kidney (455 citations), mouse hemizona assay (453 citations)*]. **PLEASE NOTE: The search must include the key word "pain" and any relevant combination thereof.** Be sure to search for all applicable terms, including the search for alternatives [*e.g. mouse heart computer model (55 citations)*]. Use the widest possible time range to include both modern and classical references.

EXPERT CONSULTATIONS

An appropriate, well documented consultation with an expert in the field of the proposed research can replace a second database search. In order to demonstrate to the IACUC the expert's knowledge of the availability of alternatives in the specific field of study, documentation of the consultation **must** include the following: (1) the consultant's name and qualifications; and (2) the date and content of the consultation.

DESCRIPTION AND JUSTIFICATION

Regardless of the sources used to search for alternatives, the written narrative should include adequate information for the IACUC to assess that a reasonable and good faith effort was made to determine the availability of alternatives to animals or procedures. If the database search or another acceptable source identifies an alternative that could be used to accomplish the goals of the proposed research; however, the investigator chooses not to use that alternative, the investigator must provide a written narrative justifying why the alternative was not used.

1. Database and Literature Search:

	Yes (X)	Date Search Conducted	Key Words/Search Strategy	Time Period Covered by Search
Databases/Computer Systems				
AGRICOLA Database (National Agriculture Libr.)	X	5/5/09	Estrogens (2115); fetal growth/development (56); gonads, testis, prematurity, pre-eclampsia, fertility, insulin resistance, sodium hydrogen exchange, etc. (15696); cesarean section (41); animal testing alternatives, animal welfare, animal models, anesthesia, pain, stress, distress, etc. (146217); papio/baboons (86), non human primates (262) = 0, 7, 7 citations/search results	1970-2009
MEDLINE Database	X	Monthly; latest on 5/4/09	Estrogens, estrogen receptor modulators (13934); gonads, testis, prematurity, pre-eclampsia, fertility, insulin resistance, sodium hydrogen exchange, angiogenesis, microvilli, placenta, etc. (145132); embryonic/fetal development (32221); pregnancy (53220); cesarean section (3338); animal testing alternatives, animal welfare, animal models, anesthesia, pain, stress, distress, etc. (1013089); papio/baboons (664); nonhuman primates (2454) = 6, 5, 7, 1 citations/search results	1966-2009
CAB Abstracts Database				
TOXLINE Database				
BIOSIS Database	X	5/6/09	Estrogens (22137); fetal growth/development (17333); gonads, testis, prematurity, pre-eclampsia, fertility, insulin resistance, sodium hydrogen exchange, angiogenesis, microvilli, placenta, etc. (74397); cesarean section (1470); animal testing alternatives, animal welfare, animal models, anesthesia, pain, stress, distress, etc. (896231); papio/baboons (1208), non human primates (22927) = 9, 9, 95, 11 citations/search results	1969-2009
Other: EMBASE	X	5/6/09	Estrogens (26539); fetal growth/development (26106); gonads, testis, prematurity, pre-eclampsia, fertility, insulin resistance, sodium hydrogen exchange, angiogenesis, microvilli, placenta, etc. (87585); cesarean section (6423); animal testing alternatives, animal welfare, animal models, anesthesia, pain, stress, distress, etc. (940629); papio/baboons (587), non human primates (2323) = 9, 24, 4 citations/search results	1974-2009
Literature and Reference Sources				
AAALAS				
Quick Biblio. Series (AGRICOLA)				
Laboratory Animal Welfare Biblio. (NLM)				
Animal Welfare Information Ctr.				

2. List any consultations with investigators in the field. (*This consult should be related to replacements, reductions, and/or refinements and not simply to the science behind the research.*)
N/A

3. Please provide a brief narrative regarding search methods utilized, but not listed above:
R01 Grant HD 13294 has been peer-reviewed and funded by NIH continuously since 1980 and funding approved for an additional 5 years in 2007. NIH-U54 grant HD 36207 was funded for 5 years in 1997 and the renewal funded in May, 2004 for an additional 5 years (2009). This grant was resubmitted in May 2008, and although approved, did not receive a score sufficient for funding and is currently in a "no cost extension". An administrative supplement to this proposal was approved for submission to the NIH to request funding through the ARRA mechanism (stimulus package) for the period 7/01/09-9/30/10. The parent U54 consortium grant will be resubmitted in spring 2011, the date the RFA for submission of U54 proposals is expected to be released by the NIH. In addition, an R01 application of studies of the impact of estrogen on fetal ovarian development (i.e. studies outlined in this IACUC application) was submitted to the NIH in Feb, 2009. All previous grant submissions have consistently been viewed as exhibiting outstanding clinical/translational relevance to the human. In addition, a search of the literature was performed in consultation with [redacted] librarian at EVMS. The databases searched included: AGRICOLA, Medline, BIOSIS and EMBASE and employed key words most notably pregnancy-fetal development, baboon and baboon-alternatives. The Medline search also employed several other key words (e.g. estradiol, estrogen antagonists, estrogen receptors). The initial search history (1966 through April, 2009) identified (depending on key word) anywhere from 2454 to over 1,013,089 results; a refined search of these hits indicated that none outlined an alternative procedure for the studies we have outlined in our protocol. Moreover, of the several manuscripts cited/abstracts printed as relevant to the search questions, the majority were publications from my laboratory. Although studies using rodents were identified, the rodent (including rat, mouse, guinea pig) is not an acceptable model for studies of human placental-fetal development. Thus, these animals do not have a fetal-placental unit, do not exhibit fetal organ system maturation as occurs *in utero* in the human (e.g. adrenal gland, ovary or testis) and exhibit unique patterns of postnatal development that impact organ system development also not typically noted in the human. Most importantly, type of placenta and thus transfer of maternal substrates across the placenta to the fetus is quite different from that in the human. In contrast, and as substantiated by the literature search, the baboon is a well-established model for studies of human pregnancy.

C. NARRATIVE: *The narrative must address the following:*

4. Provide the rationale/justification for animal use, and discuss what alternatives (e.g. *cell lines, computer simulations or artificial bodies*) were considered, and why the alternatives are not appropriate for this study's objective(s).
There is no way to perform these studies in humans and the potential impact on the development of new treatment regimens for maternal/fetal medicine are profound. For example, clinical trials in Europe show that human females born prematurely and treated neonatally with estradiol and progesterone at levels comparable to what they would have been exposed *in utero* have benefited significantly (e.g. bone maturation, neural development) over their non-treated counterparts. Epidemiologic evidence suggests that inappropriate invasion of the uterine spiral arteries may be the cause of pre-eclampsia but no one has been able to demonstrate cause:effect. Our studies suggest that too much estrogen early in pregnancy may be a causative factor and some women unsuspecting they are pregnant apparently continue to take estrogen-containing birth control pills during the very early stages of pregnancy. Other animals, such as rodents, cannot be used for such studies as they differ (e.g. placentation) considerably from the human. The baboon, like humans has a fetoplacental unit and a similar hormonal profile, placental development and metabolic machinery and fetal adrenal and ovarian anatomy, biochemistry and developmental pattern. Moreover, examination of isolated human tissue or computer simulations do not permit elucidation of the interaction between mother, placenta, and fetus in pregnancy maintenance/fetal development/parturition. As emphasized in several journals, fetal growth restriction, prematurity pre-eclampsia and infertility continue to be major health problems in the United States with annual direct costs associated with fetal immaturity alone exceeding that caused by AIDS. In humans, a poorly developed or inadequately functioning placenta results in intrauterine growth retardation/reduced neonatal birth weight and epidemiologic studies have shown that adults with low birth weight are predisposed to hypertension and reproductive dysfunction. While these clinical studies cannot provide cause:effect information, they may become more meaningful when interpreted in light of results from our *in vivo* studies in the baboon. Clearly, the experimental baboon model and the multidisciplinary collaborative approach developed by the investigators permit a necessary evaluation of the interactions essential to fetal-placental development. Thus, the results derived from the completion of this study will provide important new insight into the communication that occurs between the fetus and placenta and ultimately improve our knowledge of the regulation of pregnancy maintenance and

development of neonatal self-sufficiency and reproductive function in the human. Finally, the search of the databases outlined in Section B question 3, did not identify any alternative methods/procedures for conduct of studies to examine role of pregnancy on placental-fetal development and impact of the hormonal milieu *in utero* on physiologic/reproductive function in adulthood. Rather, the search identified manuscripts that demonstrated the value and acceptability of the baboon as a model for studies of human pregnancy.

5. Discuss the appropriateness of the species (and the animal strain, if applicable) chosen to meet the objective(s) of the study.

In the present study, we propose to continue our use of the pregnant baboon as a model to study the developmental regulation of maternal, uteroplacental, and fetal pituitary, adrenocortical/gonadal maturation and function in human pregnancy as well as impact of the intrauterine hormonal milieu on neonatal growth, puberty onset and physiologic (e.g. glucose tolerance; bone maturation) and reproductive function (e.g. ovulation; uterine function; fertility; premature menopause) in adulthood. Because the maternal, fetal, and placental units are functionally interrelated during human and nonhuman primate pregnancy, e.g. estrogen biosynthesis, they cannot be evaluated separately. Therefore, *in vitro* approaches utilizing isolated tissues do not on their own permit an assessment of the maternal-fetal-placental endocrine system. As in humans, the baboon possesses a hemochorial and monodiscoid placenta and a functional fetoplacental unit, in which the fetal adrenal gland provides the major portion of C_{19}-steroid precursors required for placental estrogen formation. Because non primate laboratory animals, e.g. the laboratory rat, do not exhibit hemochorial placentation and do not possess a fetoplacental unit for the biosynthesis of hormones such as estrogen and maturation of fetal organ systems including the adrenal and gonad occurs post-natally (i.e. extra-uterine), their applicability to the human is limited.

The qualitative and quantitative hormonal profiles exhibited in pregnant baboons are also similar in many important respects to those in pregnant women. For example, the progesterone production rate and serum progesterone concentrations are elevated during pregnancy in baboons as in women. This contrasts with other nonhuman primates, e.g. rhesus monkeys, in which serum progesterone concentrations and production rates are similar in the pregnant and nonpregnant states. An elevation in the quantities of progesterone in the peripheral circulation is essential to enable their manipulation and thus study of the regulatory factors involved. Similarities in the metabolism of progesterone during baboon and human pregnancy further support the use of the baboon for the study of steroid hormone production. Thus, the major metabolite of progesterone in women and baboons is pregnanediol, while in rhesus monkeys it is androstenedione. The concentrations and patterns of estradiol and estrone in the maternal circulation of pregnant baboons are similar to those in pregnant women, while the concentration of estradiol in rhesus monkeys at term is an order of magnitude less than in women. Corticosteroid production and metabolism also are similar in female baboons and humans. Indeed, the rate of cortisol production and excretion, type and degree of conjugation and formation of tetrahydrocortisol and tetrahydrocortisone as major metabolites are very similar in baboons and women. This contrasts with other nonhuman primates including most new-world primates (owl, squirrel and marmoset monkeys) in which serum cortisol levels and production rates are excessively high and comparable to those in humans with Cushing Syndrome.

Therefore, the baboon provides an excellent, scientifically valid model for the study of the endocrinology of human pregnancy. Finally, the 30 years of baseline data which this laboratory has obtained in pregnant baboons forms a critically important basis for the continued use of this animal model and further points to the value and peer-reviewed acceptance of the baboon for the study of the endocrinology of human pregnancy. Moreover, the search of the databases outlined in Section B question 3, did not identify any alternative methods/procedures for conduct of studies to examine role of pregnancy on placental-fetal development and impact of the hormonal milieu *in utero* on physiologic/reproductive function in adulthood. Rather, the search identified manuscripts that demonstrated the value and acceptability of the baboon as a model for studies of human pregnancy.

The numbers used are the minimum to permit collection of statistically valid and scientifically meaningful data. Sample size for comparison of means by treatment was determined by estimating the variance as from previous studies (1972-02) in my laboratory and assuming the populations are normally distributed obtained as outlined in Daniel (Biostatistics: A Foundation Analysis in the Health Sciences, 4th Ed., 1987).

6. Describe steps taken to reduce the number of animal in your study. (*e.g. replacement with in vitro procedures, refinement of experimental design, refinement of procedural techniques*).

As indicated above, there is no way to perform these studies in humans and the potential impact on the development of new treatment regimens for maternal/fetal medicine are profound. Other animals, such as rodents, cannot be used as they differ (e.g. placentation) considerably from the human. The baboon, like humans has a fetoplacental unit and a similar hormonal profile, placental metabolic machinery and fetal adrenal anatomy/biochemistry. Moreover, examination of isolated human tissue or computer simulations do not permit elucidation of the interaction between mother, placenta, and fetus in pregnancy maintenance/

fetal development/parturition. We have refined our experimental designs such that we use the same animal preparation to examine the role of estrogen on placental as well as fetal development and maternal well-being. In other words, we do a single primary experimental manipulation (e.g. injection of estrogen) and monitor the mother throughout the pregnancy (e.g. ultrasound; peripheral blood sampling for hormone and blood chemistries) and examine several aspects of placental (e.g. endovascular invasion; placental microvilli) and fetal organ system (e.g. gonad; adrenal; pituitary) development and function. Finally, the search of the databases outlined in Section B question 3, did not identify any alternative methods/ procedures for conduct of studies to examine role of pregnancy on placental-fetal development and impact of the hormonal milieu *in utero* on physiologic/reproductive function in adulthood. Rather, the search identified manuscripts that demonstrated the value and acceptability of the baboon as a model for studies of human pregnancy.

7. Will the animals be subjected to procedures that may cause more than momentary or slight pain or distress? **NOTE**: These *procedures include environmental, nutritional or behavioral modifications that increase stress, as well as chronic food or water deprivation.*

YES **(A database search is required)**

X *Complete* ***Question 8*** _____ NO (*Skip to* ***Question 9***)

8. If alternative procedures have been identified, describe the procedures below, and explain why they are not scientifically appropriate for this research project:

The search of the databases outlined in Section B question 3, did not identify any alternative methods/ procedures for conduct of studies to examine role of pregnancy on placental-fetal development and impact of the hormonal milieu *in utero* on physiologic/reproductive function in adulthood. Rather, the search identified manuscripts that demonstrated the value and acceptability of the baboon as a model for studies of human pregnancy.

9. Is the proposed study duplicative of research previously undertaken by the investigator or other scientists? **If yes, describe the duplicative nature of this project and offer scientific justification.**

No. Thus, the search of the databases outlined in Section B question 3, did not identify any alternative methods/procedures for conduct of studies to examine role of pregnancy on placental-fetal development and impact of the hormonal milieu *in utero* on physiologic/ reproductive function in adulthood. Rather, the search identified manuscripts that demonstrated the value and acceptability of the baboon as a model for studies of human pregnancy.

10. Federal regulations require a written rationale/justification for the number of animals to be used. Describe the statistical test (*e.g. power analyses and/or other rationales such as tissue collection needs and breeding efficiency*) used to determine the number of animals required to complete the proposed study, and provide the results of the test. **NOTE: The IACUC may require a consultation with a statistician.**

A statistician at EVMS was previously consulted. For example, in studies of fertility (i.e. Study 4), we calculate that with an n of 10 animals per group, there will be more than 80% power to detect a difference ($P<0.05$, Chi-square with continuity correction) in the proportion of baboon adolescents achieving a pregnancy in the untreated (90%) vs that in estrogen-suppressed (30% to 20%, respectively) baboons. For the gonadotropin studies, with n=8/group, there will be at least 80% power to detect a difference ($P<0.05$) in FSH levels that will increase serum estradiol and the level of estradiol achieved (i.e. number of follicles stimulated) between the 3 treatment groups at Ø (pooled estimate of variance) = 2.0. For analysis of the number of samples required to ascertain whether there are statistical differences ($P<0.05$) between populations in ovarian morphology/biochemical measures (e.g. follicles/0.033 mm^2; estrogen receptor mRNA/unit housekeeping gene) using analysis of variance with post hoc comparison of means by the Neumann-Keuls statistic and appropriate transformation of variables to satisfy ANOVA assumptions, there will be 82% power to identify differences between the 3 treatment groups with n=6/group (Ø = 2.0). For experiments in Study 3, statistical differences ($P<0.05$) between populations in ovarian morphology (i.e. mean ± SE follicle numbers/0.33 mm^2, oocyte microvillus number/height) and biochemical development (i.e. FSHR mRNA levels) will be determined by analysis of variance with *post hoc* comparison of the means by the Newman-Keuls statistic and appropriate transformation of variables to satisfy ANOVA assumptions. With n=6/group and pooled estimate of variance (ϕ) of 2.0, there will be at least 82% power to identify differences between the 3 treatment groups.

D. USDA PAIN CODE:

11. For each of the appropriate pain code descriptions, list the species (and the animal strain, if applicable) and the number of animals to be used each year. **Please provide the total for all three years.**

Level B

Breeding or holding colony protocols where animals are not undergoing any manipulation.

Species	Year 1	Year 2	Year 3	Total
Baboon (*Papio anubis/cynocephalus*) adult male breeders	5	→	→	5

Level C

Teaching, research, experiments or tests conducted on animals involving no or momentary/slight pain or distress (i.e., *euthanizing animals for tissues; injections, observation under normal conditions; positive reward projects, use of Acepromazine for vasodilatation in rabbits*), and **for which no pain-relieving drugs are used.**

Species	Year 1	Year 2	Year 3	Total
Baboon (Papio anubis/cynocephalus) Female adult and juvenile	30	30	30	90

Level D

Teaching, research, experiments, surgery, or tests conducted on animals involving a degree of pain or distress (*i.e., non-survival surgery, survival surgery, antibody production; subcutaneous implants, induced infections*) and **for which appropriate anesthetic, analgesic or tranquilizing drugs are used to relieve the pain and distress.**

Species	Year 1	Year 2	Year 3	Total
Baboon (*Papio anubis/cynocephalus*)	30	30	30	90

Level E

Teaching, research, experiments, surgery or tests conducted on animals involving a degree of pain or distress and **for which the appropriate anesthetic, analgesic or tranquilizing drugs are NOT used because their use will adversely affect the procedures, results, or interpretation of the teaching, research, experiments, surgery or tests. (SCIENTIFICALLY JUSTIFIED IS REQUIRED)**

Species	Year 1	Year 2	Year 3	Total
NONE				

E. STUDY PROCEDURES:

12. Please indicate all procedure(s) that will be performed in the study. **Attach all required forms.**

__X__ Non-Survival Surgery *(Complete **Attachment E, ANIMAL SURGERY**)*

__X__ Single Major Survival Surgery *(Complete **Attachment E, ANIMAL SURGERY**)*

__X__ MULTIPLE Major Survival Surgery *(Complete **Attachment E, ANIMAL SURGERY**)*

_____ Prolonged Restraint *(Complete **Attachment C, PROLONGED PHYSICAL RESTRAINT OR STRESS**)*

__X__ Collection of Tissues, Cells or Organs

_____ Adverse Conditioning

_____ Special Diet

_____ Food/Water Deprivation *(Complete **Attachment C, PROLONGED PHYSICAL RESTRAINT OR STRESS**)*

__X__ Use of Biohazards or Chemical Agents *(Complete **Attachment D, USE OF HAZARDOUS AGENTS**)*

_____ Burns or Trauma

_____ Antibody Production *(Complete **Attachment F, ANTIBODY PRODUCTION**)*

__X__ X-Rays or other Radiation

_____ Tumor Transplantation/Induction

_____ Toxicity Testing (LD-50) *(Complete **Attachment G, DEATH AS AN ENDPOINT**)*

__X__ Teaching or Training Protocol *(If checked, complete Question 12a below)*

12a. If this is a teaching or training protocol, please check all that apply:

_____ Undergraduate or graduate students

_____ Continuing education students (M.D.)

__X__ Only dead animals or tissues obtained through euthanasia by the PI to be used

_____ Demonstration (PI only performing procedures)

_____ Student involvement (students performing/assisting in procedures)

_____ Use within a Biomedical Sciences Course (ID #/Name: _____)

_____ Other (**Explain below**):

F. RESEARCH DESIGN:

13. In generating the research design, note that the reviewers are scientifically knowledgeable; however, they may not be experts in your specific field of study. Please provide a brief (one or two paragraphs) overview of the project design and how each experimental goal relates to the project design. The descriptions should provide a sequential overview of all procedures and should account for each subject (by experimental group). The overview should be followed by a chronological description of all experimental procedures related to the care and use of the animals. **The use of tables and flow charts to organize the procedures, numbers of animals, and schedules is recommended.** Do not paste in method sections from grant applications or journal articles. Do not include methods pertaining to *in vitro* work, unless it applies to the care and use of animals. For each animal or experimental group, provide information on the duration of each procedure (*i.e. fluid or tissue collections, methods, sites, volumes/weights, frequencies, etc.*) and the total time from initial contact to completion. **Although procedures involving drug manipulations and surgery are detailed in other sections of this form, their application in the research design should be stated here. Any procedures not covered in later sections of this form must be completely detailed in this section.**

By reading only this section of the *Initial Review Form*, the IACUC should be able to clearly determine each experiment being performed on each individual animal.

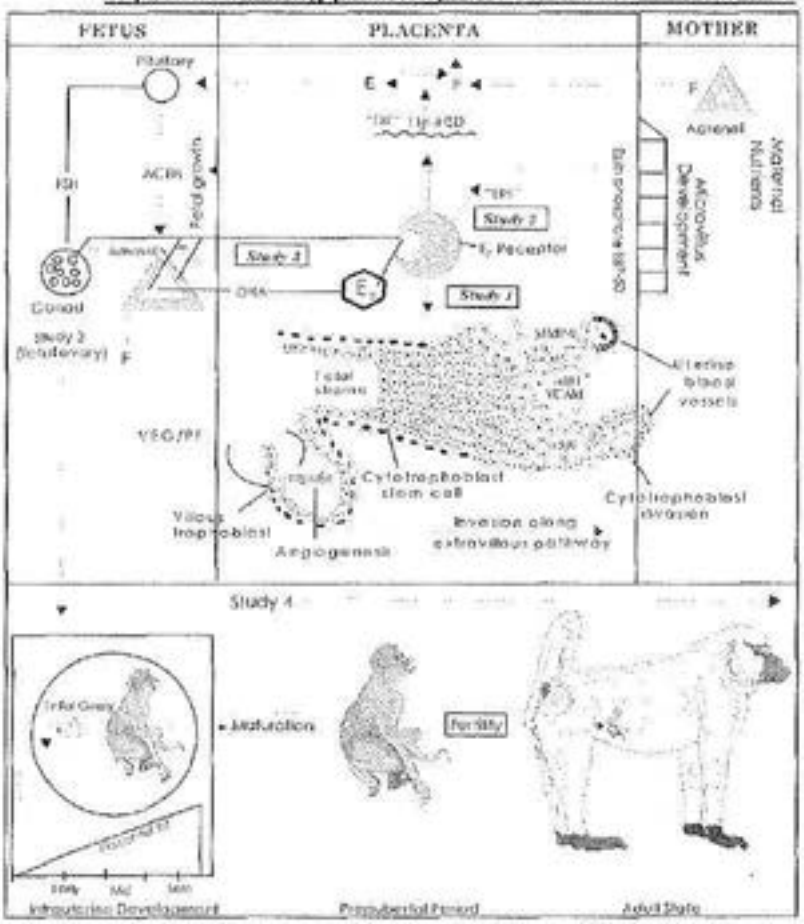


Fig 1. Outline of the study of regulation of placental (Studies 1 & 2) and fetal adrenal and gonadal (Studies 1 & 3) development by estrogen (E_2) during primate pregnancy and impact (Study 4) on reproductive function in adulthood.

EXPERIMENTAL TREATMENT GROUPS (*See Fig 1 for outline of experimental mission*):

1. pregnant baboons studied 0, 2, 6 or 12h following a bolus injection of estradiol on day 54 to examine acute effects of estrogen (i.e. mechanisms); n = 12 pregnancies/group; ***total pregnancies = 48.*** More animals are needed here since size of placenta is small and tissue is limited [Studies 1 and 2; see Fig 1]. Majority of studies performed at Maryland.
2. pregnant baboons studied on day 60 following treatment with nothing or estradiol (or androgen precursor androstenedione) on days 25-59 to examine role of estrogen on placentation, angiogenesis and vascular invasion; n = 6 pregnancies/group, ***total pregnancies= 12*** [Study 1]. Majority of studies performed at Maryland.
3. pregnant baboons studied on day 160-170 following treatment with nothing or estradiol (or androgen precursor

androstenedione) on days 25-59 to examine impact of early estrogen on fetal/placental growth; n = 6 pregnancies/group, ***total pregnancies= 12*** [Studies 1, 2 and 3 Majority of studies performed at Maryland.

4. pregnant baboons studied on day 160-170 following no treatment or daily maternal treatment with Letrozole (reduces estrogen production chronically), or Letrozole and estradiol beginning on day 100. n = 6 pregnancies/group; ***total pregnancies = 18*** [Studies 2 and 3];
5. pregnant baboons studied on day 110 of gestation and treated on days 100-109 with nothing or Letrozole to reduce estrogen short-term. Fetus/placenta delivered on day 110. n = 6 pregnancies/group; ***total pregnancies = 12*** [Studies 2 and 3]
6. pregnant baboons studied on day 100 untreated; placenta, fetal adrenal and gonads studied *in vitro* using short term culture; n = 6 pregnancies; ***total pregnancies = 6*** [Studies 2, 3]
7. pregnant baboons studied on day 110 of gestation and treated on days 100-109 with Letrozole to reduce estrogen short-term and injected with estradiol on day 108/109 and fetus/placenta delivered 6 (n = 6), 12 (n = 6), 24 (n = 6) or 48 (n = 6) hours after first injection of estrogen to examine ability/mechanism of estrogen to restore fetal/placental function. Another group of 6 animals will be treated with Letrozole on days 100-109 and injected with oil vehicle on days 108/109 and fetus/placenta delivered 48 hours after first injection of oil (i.e. control for estradiol; study numbers limited to only 48h). ***total pregnancies = 30*** [Studies 2 and 3]
8. pregnant baboons studied on day 140 of gestation and treated on days 120-140 of with letrozole to reduce estrogen production by the placenta. On day 140, a single fetal injection (via ultrasound guided probe) with 17β-estradiol (1 μg in 0.2 ml sesame oil). Delivery of baboon fetus via cesarean section 2, 12 or 24 hours after injection of estradiol or 24 h after injection of vehicle (saline:ethanol) ***(total pregnancies = 24)*** [Studies 2, 3].
9. pregnant baboons studied on day 140 of gestation and treated on days 120-140 of gestation with nothing, letrozole to reduce estrogen production by the placenta or with letrozole and estradiol to restore estrogen. Delivery of baboon fetus via cesarean section on day 140. ***(total pregnancies = 18)*** [Studies 2, 3].
10. pregnant baboons studied on day 165 of gestation and treated on day 140-165 with Letrozole to reduce estrogen production by the placenta. On day 165, a single fetal injection with 17β-estradiol (1 μg in 0.2 ml sesame oil (via ultrasound guided probe). Delivery of baboon fetus via cesarean section 0, 2, 4 or 6 hours after estradiol injection. ***(total pregnancies = 24)*** [Study 3]
11. pregnant baboons studied on day 165 of gestation and treated on day 140-165 of gestation with Letrozole to reduce estrogen production by the placenta. On day 165, fetal injection (via ultrasound guided probe) with either; a) vehicle dimethylsulfoxide (1% DMSO in saline; total volume 0.5 ml), b) Neomycin (10-50mg/kg BW) in saline, c) Wortmannin (1.4 mg/kg BW/0.5 ml DMSO), d) U0126 (100 mg/kg BW), or e) Y-27632 (1.4mg/kg BW in DMSO) followed one hour late by a single injection of 17β-estradiol (1 μg in 0.2 ml sesame oil). Delivery of fetus via cesarean section 6 hours after estradiol injection. Preliminary work indicates that Wortmannin and U0126 may not be required ***(total pregnancies = 18)*** [Study 3].
12. neonates born on day 170 delivered to mothers untreated or treated *in utero* with Letrozole or Letrozole and estradiol n = 10 pregnancies/group; ***(total pregnancies = 30).*** Babies are examined daily by Dr. Pepe's staff and weaned from their mothers at 12 months of age. All female babies are studied at EVMS and males shipped to the University of Maryland for study. (Female babies born at Maryland shipped to EVMS). At 2-4 week intervals beginning at approximately 6 months of neonatal age, mothers are sedated with ketamine and all neonates removed, sedated with ketamine, weighed, a gross physical examination performed by PI staff and blood samples (<1 ml) obtained for determination of blood chemistries (e.g. Na, K, glucose) and hormone profiles (cortisol, androgens, Estradiol; insulin, prolactin, growth hormone, ACTH). Intravenous glucose tolerance testing and radiograph (X-ray) of the epiphyseal plate (wrist) are performed at 6 month intervals as outlined below. Females originally born at EVMS or arriving from the University of Maryland at age 12 months or greater are evaluated at EVMS. After onset of puberty defined as onset of regular menstrual bleeding and which usually occurs at 36-48 months of age, female adolescents are studied to determine adequacy of ovarian function and uterine responsivity. Briefly, female adolescents are sedated with ketamine and blood samples (1-2 ml) obtained daily during two subsequent menstrual cycles (cycle one animal studied; cycle two animal not studied; cycle three animal studied) for subsequent measurement of Estradiol (ovarian follicular phase function), progesterone (ovulation/ovarian luteal phase function) and LH and FSH (pituitary function/drive). Ovarian follicle size and uterine endometrial growth are determined by non-invasive ultrasound/or transvaginal probe during the follicular and luteal phases of the 34-36 day menstrual cycle (a total of 8 ultrasounds are performed e.g. before, at and after ovulation). Baboons, now adults are then examined for fertility studies, i.e. ability to achieve (mated with males of proven fertility) and maintain pregnancy and deliver a healthy neonate. After fertility testing has been completed, animals are hemi-ovariectomized (as outlined below) to assess ovarian follicle reserve/ biochemistry/health) and then following insertion of indwelling cannulas (as outlined below) challenged with LH and FSH (Gonadotropin Challenge Test; as outlined below) to assess ovarian response to pituitary gonadotrophin. [Study 4].

Overall Total pregnancies: n = 252. This research program continues to function as a collaborative effort with colleagues at the University of Maryland as has occurred over the past 30 years. Thus, the majority of *in vivo* studies outlined above as 1-3 (total pregnancies = 72) will be performed at the University of Maryland while those in

categories 4-12 will be performed at EVMS; 50% of animals in category 12 will be derived/studied at both Institutions. Tissue samples will be analyzed at both Institutions. Therefore, ***a total of 180 pregnancies are required at EVMS. As these are ongoing studies and experiments previously approved by IACUC, the overall total number of experiments (i.e. pregnancies) that still need to be completed in each category approximate 65%. Thus a total of approximately 117 pregnancies are required (23/year over a 5 year period) to complete the objectives outlined.*** Based on our experience and multiple use of baboons (control, estrogen suppression, estrogen treatment etc), a population of 26 adult female and 3 adult male baboons is required to meet the objectives of this study. Because multiple surgeries are limited, we also have determined that we need to purchase at least 3 and up to 5 adult female baboons yearly to "turn over" the colony.

As indicated on page 15, studies 1-3 using adult pregnant baboons will be performed almost exclusively using animals housed at the University of Maryland. Maryland will ship tissues (e.g. placenta for study of microvilli development) to EVMS for analysis. However, because this is a collaborative program and there may be an occasion (e.g. following review of a submitted manuscript) where experimentation may need to be performed here at EVMS, I have included this in our protocol. However, the impact on adult animals housed at EVMS is virtually nil. Consequently, the majority of adult animals housed at EVMS will be used for studies 4-11. In these experiments, all fetuses will be delivered at a specified stage of gestation and thus will not be raised to adulthood. As indicated in the paragraph above, to complete these studies **we need 180 x 0.65 or 117 pregnancies, not 117 animals** since we are requesting and have been approved to perform multiple survival surgery which limits the number of animals. To further limit the number of animals required, we perform an amniocentesis at approximately day 90 of gestation to determine fetal sex, which permits us to determine which protocol an animal is assigned (e.g. determine impact of estrogen deprivation on fetal ovarian development at term). The study of placental and other organ system development is not related to sex of the fetus. In study 11, in which fetuses will be delivered and reared to adulthood, we also know the fetal sex. However, because this is a consortium grant, we felt it was important to have animals exposed *in utero* and reared at both institutions to more appropriately examine impact of the hormonal milieu on physiologic function/reproduction in adulthood. In other words, we anticipate obtaining comparable results in animals reared in two different albeit experimentally controlled environments. Thus males and females born at EVMS will be studied as outlined until age 12 months and then males shipped to Maryland and females born at Maryland shipped to EVMS.

ANIMAL HUSBANDRY:

Juvenile and adult female baboons are housed in USDA regulated cages. Socialization and behavior is monitored by PI staff and CompMed jointly. When possible, female baboons are socialized and pair housed with compatible females. Some pairs are fully open allowing free interaction. In some cases as a result of aggressive behavior causing injury, two females are 'partial paired' meaning they do not have continues free interaction but are restricted allowing tactile contact and socialization on a limited level. Cycling adult female baboons are paired with male baboons for breeding purposes 5 days prior to ovulation as determined by perineal turgescence or sex skin swelling. Successful breeding resulting in pregnancy is confirmed by ultrasound on day 25 (day 0 = day of ovulation) and/or lack of menstrual cycle and halted sexual skin swelling.

SURGERIES:

[1] ***Cesarean section***: On day 54, 60, 100, 110, 140 or 165 of gestation, baboons are briefly restrained in home cage via squeeze mechanism, injected with ketamine (10 mg/kg), intubated, anesthetized with isoflurane/oxygen and vitals (e.g. heart rate, blood pressure) monitored by CompMed staff. A 22g catheter is placed in the brachial vein and IV fluids administered (Lactated Ringers Solution or 0.9% saline). A second catheter is placed in the saphenous vein using a 19g catheter 24inches in length and 5% dextrose fluids administered. The animal's abdomen/surgical site is shaved and scrubbed with alcohol and Betadine solution. The animal is draped using sterile technique. Blood samples are obtained from the mother at '0' time, mid procedure and post placental delivery via saphenous catheter. A vertical mid-line incision is made using a 10blade. Once the abdomen is open, the uterus is externalized and examined for placenta location. Mother's ovaries are examined for evidence of ovulation/corpus luteum. Two to four uterine vein blood samples are taken (3-5 ml) using a 23g needle. Fetal crown is localized and a horizontal incision is made in the uterus to remove the fetus. Amniotic fluid is recovered using a syringe. Once the fetus is carefully exteriorized, umbilical vein and artery samples are taken using a 23g butterfly set (< 1 ml on day 54-60; 1-2 ml on day 100 and 4-6 ml on day 170) for hormone, steroid and blood gas analysis. The umbilical cord is double clamped to ensure the safety of the mother; the fetus is then euthanized by injecting via the umbilical artery Fatal Plus euthanasia solution. After the fetus expires (no heart beat), the cord is cut, placenta is delivered. Segments of the placenta and the fetal adrenal, hypothalamus, pituitary gland, lungs, kidneys, liver, skeletal muscle, pancreas, subcutaneous and visceral fat, and gonads are collected, portions fixed in formalin or snap frozen for subsequent immunocytochemical-biochemical/mRNA determinations. The uterus is cleaned and closed using 2-0 PDS II suture. The uterus is manually messaged to stimulate contractions and shut down bleeding. Once closed, the uterus is rinsed with sterile saline and placed back in the abdomen. The abdomen is then rinsed with sterile saline to remove any blood clots. Prior to closing the abdomen, a small 10-15gram sample of visceral/abdominal fat is ligated and removed for RNA analysis. The abdomen is closed by three layers; the first

layer (peritoneum) is closed using 2-0 PDS II simple interrupted stitch. If present, a second layer (fascia) is closed using 2-0 Dexon II suture. If a clear fascia layer is not present then a SQ layer is closed using a continuous stitch. Finally, bupivacaine is applied topically prior to closing the third and final layer. The skin is closed using 3-0 PDS II. Vet-bond adhesive glue is applied to the incision line once skin is closed. The mother is injected with 100mg Iron Dextran IM for iron supplementation. Buprenorphine 0.03mg/kg and Ketoprofen 2mg/kg is given IM during immediate recovery. Isoflurane is shut off and animal is monitored until swallowing is present. Once able, the animal is extubated and continues to be monitored in home cage until fully upright and responsive to stimuli. Immediate post-op monitoring is done by Com Med staff using procedures outlined in the EVMS Post-Operative Care Guidelines Manual and in Attachment E below. PI staff is also present during immediate recovery. In selected experiments, the fetus is delivered live either spontaneously (on day 184) or by cesarean section on day 170 and reared to adulthood (also please see experimental treatment group #12; page 15).

[2] ***Hemi-ovariectomy***: Adolescent baboons from experimental group #12 are briefly restrained in home cage via squeeze mechanism, sedated with ketamine (10mg/kg), intubated, anesthetized with isoflurane/oxygen and vitals (e.g. blood pressure) monitored by CompMed staff. A 22g catheter is placed in the brachial vein and IV fluids administered (Lactated Ringers Solution or 0.9% saline). A second catheter is placed in the saphenous vein using a 22 g or 19g catheter 24inches in length and 5% dextrose fluids administered. The animals' abdomen/surgical site is shaved and scrubbed with alcohol and Betadine solution. The animal is draped using sterile technique. After making a small abdominal incision a retractor is put in place, lap sponge can be used if needed for a clearer view, ovaries are located. Once the ovulation site is located, the ovarian ligament and vasculature is identified, clamped and cauterized and the ovary removed. Once removed, clamps are carefully removed and ligament/cautery site observed for bleeding. Abdomen is rinsed with sterile saline. Abdominal layers are closed (peritoneum, fascia/SQ and skin) using 2-0 PDS and 2-0 Dexon. Bupivacaine applied topically prior to skin closure. Vet-bond skin adhesive applied to the incision site once closed. Buprenorphine 0.03mg/kg and Ketoprofen 2mg/kg is given IM during immediate recovery. Isoflurane is shut off and animal is monitored until swallowing is present. Once able, the animal is extubated and continues to be monitored in home cage until fully upright and responsive to stimuli. Immediate post-op monitoring is done by Com Med staff using procedures outlined in the EVMS Post-Operative Care Guidelines Manual and in Attachment E below. PI staff is also present during immediate recovery. Animals will be treated postoperatively with analgesics and recovery monitored as outlined in the Attachment E below. *In vivo* responsivity of the remaining ovary to pituitary gonadotropin will be initiated no sooner than 6-9 months later and after animals have exhibited at least three consecutive normal menstrual cycles. Gonadotropin challenge studies will require implantation of indwelling cannulas for constant infusion of pituitary hormones over an extended period.

[3] ***Gonadotropin Challenge Test / Implantation of indwelling cannulas***: Experimental group #12 - Beginning on experimental day 0, (14-21 days post hemi-ovx) baboons will be injected IM every other day over a ten day period with a GnRH antagonist (e.g. leuprolide/Antide) to suppress endogenous pituitary gonadotropin secretion. Blood samples (3 ml) will be obtained under ketamine sedation from a saphenous vein at two-day intervals and serum estradiol and progesterone levels determined to confirm suppression of pituitary and thus ovarian function (i.e. levels of estradiol/progesterone should be negligible). On experimental day 10 or once suppression is reached, baboons will be sedated with ketamine, anesthetized with isoflurane/oxygen, intubated and an IV catheter placed in the brachial vein for IV fluid administration. Vitals are monitored (pO_2, blood pressure, HR, resp, and blood gas). Neck and back (between the shoulder blades) are shaved and scrubbed for sterile procedure. A small incision is made in the neck to expose the right jugular. A cannula of relatively small length is inserted into the right jugular vein and secured using suture. Implantation into the jugular vein permits use of a cannula smaller in length than if inserted into the femoral vessels. The cannula/PE tubing (0.030") will then be tunneled subcutaneously by way of sterile trochar to the scapular region and connected to ALZET osmotic minipump (Model 2ML1; 5.1 cm by 1.4 cm length/width; 5.1 grams weight; 6.5 ml volume) designed to deliver human LH or FSH (4.8 µg hLH/kg BW/day and 480 ng hFSH/kg BW/day) which is dissolved in sterile saline containing penicillin/streptomycin. A small incision is made in the scapular area to connect the pump to the PE tubing. Pump is implanted just under the skin. The exact dosage of hLH and/or hFSH infused may need to be altered slightly depending upon the LH and FSH level achieved in blood samples obtained 24 hrs after onset of infusion and is the reason human FSH (and LH) are used in this study since we can measure hLH and hFSH levels rapidly using our Diagnostic Immulite Automated RIA system. Both incisions are closed using sterile technique. Bupivacaine applied topically prior to skin closure. Vet-bond skin adhesive applied to the incision site once closed. Buprenorphine 0.03mg/kg and Ketoprofen 2mg/kg is given IM during immediate recovery. Isoflurane is shut off and animal is monitored until swallowing is present. Once able, the animal is extubated and continues to be monitored in home cage until fully upright and responsive to stimuli. Immediate post-op monitoring is done by Com Med staff using procedures outlined in the EVMS Post-Operative Care Guidelines Manual and in Attachment E below. PI staff is also present during immediate recovery. Animals will be treated postoperatively with analgesics and recovery monitored as outlined in the Attachment E below. After 7-10 days of infusion, (i.e. experimental day 17-20), the pump will be replaced with another Model 2ML1 pump containing the same amount of LH and a dose of FSH 1.25 times greater than that ultimately delivered during the previous 7 day period (i.e. approximately 600 ng FSH/kg BW/day). Baboons will be sedated with ketamine, anesthetized with isoflurane/oxygen, intubated and an IV catheter placed in the brachial vein for IV fluid administration. Vitals are monitored (pO_2, blood pressure, HR, resp, and blood gas). Scapular area scrubbed and

prepped for ALZET pump replacement. Once the new pump is in place under the skin, Bupivacaine applied topically prior to skin closure. Vet-bond skin adhesive applied to the incision site once closed. Ketoprofen 2mg/kg is given IM during immediate recovery. Isoflurane is shut off and animal is monitored until swallowing is present. Once able, the animal is extubated and continues to be monitored in home cage until fully upright and responsive to stimuli. Immediate post-op monitoring is done by CompMed staff using procedures outlined in the EVMS Post-Operative Care Guidelines Manual and in Attachment E below. PI staff is also present during immediate recovery. Animals will be treated postoperatively with analgesics and recovery monitored as outlined in the Attachment E below. Blood samples (3-5 ml) will continue to be collected during the study period under ketamine sedation and after 7 days of treatment with the larger dose of FSH (i.e. experimental day 24) the animal will be euthanized and the remaining ovary collected for histological/biochemical evaluation.

***Experiments to determine hormone production rates*:** We will use intravenous constant infusion of differentially labeled (tritium, carbon 14) substrates to determine hormone production rates as well as transfer of maternal substrate across the placenta to the fetus. These experiments will be performed on day 160-170 of gestation in pregnant baboons untreated or treated with Letrozole without/with estradiol benzoate (Groups 4 and 6 above).Briefly the animal will be prepped for surgery as outlined above. An IV injection of the isotope will be delivered at time '0'. Blood samples will be taken from the saphenous catheter at 50, 55 and 60 minutes. C-section delivery of fetus and placenta will follow surgical outline as above. In addition, adolescent baboons at approximately age 60-84 months of age will undergo IV infusion of differentially labeled (tritium, carbon 14) substrates to determine hormone production rates while under Ketamine sedation. Briefly, animals will be restrained in home cage, sedated with 10mg/kg ketamine, transferred to procedure room and monitored for blood gas, O_2 output, BP, HR and respiration. An IV line established in the saphenous and brachial vein. An infusion of the isotope will be delivered in the brachial and blood samples taken at '0', 50, 55 and 60 minutes from the saphenous. Animal will recover, returned to home cage and be monitored to recovery stage 0. Urine is collected for 3 days and disposed of by radiation safety as outlined in EVMS' policy for Radiation Safety item 14 Animal Procedures. The constant infusion procedure is non-invasive and allows determination of steroid hormone production rates by the adrenal gland without having to do a surgical intervention (i.e. cannulation or sampling the adrenal vein). (Group 11 above).

Intravenous glucose tolerance test (IVGTT): An IVGTT will be performed in adolescent baboons at 12, 24, 30, 36, 42, 48, 54, 60, 66 and 72 months of age (i.e. before, during and after onset of puberty which occurs at approximately age 40 ± 1 month). Briefly, baboons (4-12 kg body weight) are fasted overnight, sedated at 0900 h with ketamine (10 mg/kg), a catheter inserted into a peripheral saphenous and an antecubital vein and a 250ml 0.9% sterile saline drip with 500mg ketamine is initiated. Two initial base line blood samples are taken and blood glucose and blood gas levels determined. At experimental time zero, a bolus of glucose (0.25 grams/kg BW; 0.05 ml 50% dextrose/kg BW; Abbott Laboratories)is injected into the antecubital vein. Blood samples (< 1.0 ml) are collected into heparinized syringes from the saphenous catheter at 0, 1, 3, 5, 10, 20, 40, 60 and 90 mins and 0.1 ml used to determine blood glucose. The remainder of the sample is kept on ice and serum subsequently assayed for insulin/C-peptide by RIA. During the experiment, the animal is monitored for BP, HR, respiration, and warmed on a heating pad and heated table. At completion of the experiment, animal is given 1cc (100mg) iron dextran IM. Animals are returned to their cages and monitored to recovery to stage 0.

X-ray of Juvenile Growth Plate: An x-ray of the growth plate in the wrist will be performed in adolescent baboons at 12, 24, 30, 36, 42, 48, 54 and 60 months of age (i.e. before, during and after onset of puberty. Briefly animals are fasted overnight, sedated with ketamine (10 mg/kg), taken into x-ray facility and with the assistance of Comp Med, an x-ray is taken. Animals are returned to their cage and monitored to recovery to stage 0.

Studies of fetal ovarian development following injection of fetus with estradiol and/or pharmacologic agents: Maternal baboons with confirmed female progeny will be treated daily with letrozole between days 120 and 140 or 140 and 165 of gestation. On day 140 or 165, maternal baboons (fasted overnight), will be sedated with ketamine (10 mg/kg BW), and lightly anesthetized with halothane (via mask) and an acute experiment will be conducted in which the fetus will receive on day 140: a single IM injection (23g needle, ultrasound guided) of 17β-estradiol (1 μg/kg BW) or saline:ethanol vehicle and the fetus delivered 2, 12 or 24h later (12 h experiment stared in late PM) as outlined above; for day 165 studies, the fetus will be administered a single injection IM early in the AM with either 1) vehicle (1% DMSO in saline or saline) 2) Neomycin (10-50mg/kg BW) in saline, 3) Wortmannin (1.4 mg/kg BW/0.5 ml DMSO), 4) U0126 (100 mg/kg BW), or 5) Y-27632 (1.4mg/kg BW in DMSO) followed one hour later by a single injection of 17β-estradiol (1 μg in 0.2 ml sesame oil). During this time, the mother will be under isoflurane/O_2 anesthesia via cone mask and monitored for HR, PO_2 level and BP. Also, an IV catheter will be in place for the hour to administer fluids (5% dextrose solution). Following fetal injections, maternal baboons are then returned to their cages and monitored to recovery. All postoperative monitoring will be recorded in the animals' record and will follow established EVMS monitoring guidelines. Maternal baboons will remain fasted except for water *ad libitum,* and will be re-sedated with ketamine, and undergo under isoflurane anesthesia a standard cesarean section 6 hours later to obtain the fetal ovaries for comprehensive molecular studies.

14. **Adverse Effects: Monitoring and Management**:

14a. In detail describe the potential adverse effects of each experimental procedure or agent administered to animals. For each item, include a statement detailing how the adverse effects would be clinically managed, should they occur.

Ketamine: IM injection for chemical restraint prior to blood sampling, ultrasound examination, IVGTT, or surgery. Ketamine is a dissociative anesthetic. Animals can develop tolerance and require increasing doses for effective sedation. Adverse effects can include nerve damage (if injection is improperly placed) and decreased appetite for up to 48 hours post-sedation. Also, Ketamine can have a long term effect on kidney function. When possible, the lowest dose is used and each animal is evaluated on its responsive behavior to the drug. Also, blood gas analysis is done bi-weekly to monitor BUN levels. All changes in weight, appetite or blood chemistry are reported to The veterinary staff (veterinary technicians and/or veterinarian).

Follicle stimulating hormone (FSH) and luteinizing hormone (LH): these structurally-similar hormones are administered via ALZET pump placed beneath the skin for constant infusion to stimulate ovarian follicular development. Adverse effects: none anticipated, no rejection issues to date.

Buprenorphine: IM administration for pain relief. Adverse effects: can cause respiratory depression and should be used with caution in animals with impaired cardiovascular function or liver problems. No side effects have been reported in our study.

Glycopyrrolate: IM administration as a preanesthetic prior to surgery. Adverse effects: dry mouth, drowsiness. Adverse effects are rare with single administration as used in this protocol but may include gastrointestinal, urinary, central nervous system, and cardiovascular side effects; The veterinary staff (veterinary technicians and/or veterinarian) will be notified if side effects occur.

Isoflurane: inhaled to maintain proper plane of anesthesia during surgical procedures. Adverse effects: none anticipated. Animals are closely monitored during surgery. If the animal moves, shows eye movement, has increased jaw tone, or shows a rapid increase in heart rate or blood pressure, then isoflurane administration will be increased. Possible side effects can be hypotension, dose-dependent respiratory suppression, cardiodepression and GI effects (nausea, vomiting, ileus).If animal shows a decreased heart rate, decreased blood pressure, or pale gum color with reduced capillary refill time (CRT), then isoflurane administration will be decreased, along with decreased intravenous fluid flow rate.

Ketoprofen: IM administration for pain relief. Long term administration can cause ulceration of the GI tract and GI bleeding; more rarely kidney damage and other bleeding disorders can occur. Adverse effects are not anticipated with the short-term administration described in this application.

Abdominal surgery: general risks associated with abdominal surgery include blood loss, infection, and adhesions. Undetected blood loss will be prevented by ensuring hemostasis before closing surgical incisions. All animals are monitored during the post-operative period (as defined by IACUC policy) for signs of internal bleeding (vasoconstriction and resulting loss of color of digits/extremities, lethargy, dehydration). Infection will be minimized by use of sterile equipment and supplies, disinfection of the incision site, performance of surgery in a dedicated surgical suite, and use of aseptic technique during the procedure. Infection rate is minimal to none in 300+ survival surgical procedures performed by the PI/PI staff at EVMS. The Veterinarian will be consulted if unusual redness, swelling, or discharge is noted at the incision site. Adhesions will be minimized by gentle manipulation of internal organs and lavage of the abdominal cavity with warm saline to remove clotted blood before closing surgical incisions.

Ovariectomy: unilateral ovariectomy results in an animal which maintains normal menstrual cyclicity; bilateral ovariectomy will yield an infertile animal. While generally healthy, ovariectomized animals may experience physical changes associated with hormone withdrawal at menopause, including decreased bone density, weight gain, and urinary incontinence.

Fetal injection: general risks associated with the injection are spontaneous abortion or stress to the fetus. At time of injection, the fetus is localized using ultrasound, change in HR is monitored. Injection is administered to rump area to minimize stress. Any distress noted will be discussed with the Veterinarian.

Amniocentesis: general side effects are stress to the fetus and possible abortion. A clear area free of fetus and placenta is localized and a small sample (± 6ml) of amniotic fluid removed. Fetal HR is checked. Any distress noted will be discussed with the Veterinarian.

IVGTT: side effects are minimal. Possible short term anemia and depreciated appetite from ketamine. Animals are given Iron Dextran injection at completion of experiment and supplemented with children's vitamin containing iron. Also, food intake following experiment is monitored.

X-ray: exposure to radioactive waves. Whole body exposure is minimal and should pose no adverse effects.

Bupivacaine: administered topically at close of surgery to the incision site. Numbing agent to relieve

pain. No expected side effects.

Letrozole: Administration of Letrozole alone (i.e. without concomitant administration of estradiol) lowers estrogen levels by >95%. When Letrozole treatment is initiated on day 100 and estrogen suppressed, approximately 10% of the baboons abort without any complications (vaginal bleeding visible); the products of conception may or may not be visible in the cage. In this case, the study is terminated, the animal watched closely over the next few days to ensure that bleeding has stopped, appetite is not depreciated and behavior is normal. In another subset of animals (approximately 10%), there is a sudden onset of seizures approximately 25-30 days into the Letrozole treatment. Animals are typically found lying down (comatose) in their cage early in the morning suggesting that seizure(s) most likely occurred overnight or very early that morning. In animals that have seized, we believe it is important to intervene at time of discovery since it is our impression that the longer the animal is left comatose, the more difficult it is to revive the animal. The following protocol seems most relevant to implement and to have been a success in the past:

- Animals which are stuperous (unsteady on their feet but conscious) will immediately be given oral juice/sugar treatment in form of frozen juice or piece of orange or candy to elevate blood glucose levels. If animal is non responsive or progresses to seizing or unconscious state the following will be implemented:
- The animal is removed from its cage and taken to the treatment room. If light sedation is required for safe transport, a small dose (5 mg/kg BW-IM) of ketamine will be administered.
- Blood gas (pO_2, pCO_2, pH etc) and glucose will be determined using I-Stat analyzer (results in 2 mins).
- Animal will be placed on O_2 at 2L/min via cone mask and body temperature recorded and maintained with heating pad and warm IV fluids.
- A catheter will be placed in an antecubital or saphenous vein and if blood glucose levels are below 50 mg/100 ml, a 5 ml bolus of 50% Dextrose in Lactated Ringers (1:1) will be delivered over a 5 min period, followed by a 5% Dextrose drip until the animal responds (glucose normalized). Adjustments to normalize pH (e.g. sodium bicarbonate) may also be required.
- If animal does not appear to respond to the above, a saturated solution of magnesium sulfate (10 ml) will be injected IV as this drug has been shown to alleviate seizures in eclamptic women.
- Once responsive, the animal is returned to its cage and monitored throughout the day.
- The animal will be removed from the protocol and will most likely abort. If the latter does not occur, the animal will be permitted to go to term and the fetus delivered by cesarean section at the end of treatment.
- Finally I want to reiterate that of the 10% that do seize, approximately 35% succumb. It is our impression that these are most likely the animals that exhibited a seizure during the night. We have identified an apparent window between 120-140 days gestation when the seizures are most likely. We (PI staff) have been evaluating changes in glucose levels and blood gases as a way to determine if the seizures will occur. Comp Med personnel, as well as my staff, are aware of this possible linkage and are attempting to resolve this problem. We do want to point out that regardless of when an animal seized, we still will employ the protocol outlined above (i.e. response to question 15a).
  In addition; decreased appetite can be seen during late treatment with Letrozole. Animal's gums can become swollen making hard biscuit consumption difficult. In this case, affected animals are given biscuits softened soaked in Ensure or Boost to assist in palatability and ensure necessary nourishment. If an animal continues to exhibit inappetence despite this treatment or experiences weight loss, the veterinarian will be consulted.

Estradiol: SC injection, IV bolus. No adverse effects anticipated.

Ovine Prolactin: SC injection to the mothers treated with Letrozole to stimulate prolactin production for nursing the neonate. It is possible that prolactin may induce spontaneous delivery although this is not an adverse event and has not been seen in our study. It is also possible that milk production will not be sufficiently restored.

Leuprolide(GnRH antagonist): SC injection, This medication may cause nausea, vomiting, hot flashes, night sweats, bone pain, swelling of feet and ankles, headaches or difficulty urinating. Animal will be monitored for signs of edema, decrease urination or apparent pain or discomfort.

^{3}H-cortisone: IV infusion, side effects are minimal. Depreciated appetite. Animal will be monitored for prolonged change in eating habits. If animal continues to exhibit in appetence, animals are given biscuits softened soaked in Ensure or Boost to assist in palatability and ensure necessary nourishment. If an animal continues to exhibit in appetence despite this treatment or experiences weight loss, the veterinarian will be consulted.

^{14}C-cortisol: IV infusion, side effects are minimal. Depreciated appetite. Animal will be monitored for prolonged change in eating habits. If animal continues to exhibit in appetence, animals are given biscuits softened soaked in Ensure or Boost to assist in palatability and ensure necessary nourishment. If an

animal continues to exhibit in appetence despite this treatment or experiences weight loss, the veterinarian will be consulted.

^{3}H-dehydroepiandrosterone: IV infusion, side effects are minimal. Depreciated appetite. Animal will be monitored for prolonged change in eating habits. If animal continues to exhibit in appetence, animals are given biscuits softened soaked in Ensure or Boost to assist in palatability and ensure necessary nourishment. If an animal continues to exhibit in appetence despite this treatment or experiences weight loss, the veterinarian will be consulted.

^{14}C-dehydroepiandrosterone sulfate: IV infusion, side effects are minimal. Depreciated appetite. Animal will be monitored for prolonged change in eating habits. If animal continues to exhibit in appetence, animals are given biscuits softened soaked in Ensure or Boost to assist in palatability and ensure necessary nourishment. If an animal continues to exhibit in appetence despite this treatment or experiences weight loss, the veterinarian will be consulted.

Estradiol-17β: IM to fetus, side effects at such low dose are minimal. Stress from the manipulation is more likely than from the agent itself. Fetal HR is monitored during the injection and any observed change is noted and the Vet is consulted. No such incidence has occurred

U0126: IM to fetus. There are no expected side effects from this agent. Any stress noted to the fetus will be conveyed to the veterinarian.

Wortmannin: IM to the fetus. There are no expected side effects from this agent. Any stress noted to the fetus will be conveyed to the veterinarian.

DMSO: IM to fetus. Used as delivery agent, no adverse effects at this dose.

Neomycin: IM to fetus. Neomycin is can be a nephrotoxic. The fetus is non-survival so this is not a concern. At this low dose there should be no adverse affect to the dam.

Y27632: IM to fetus. There are no expected side effects from this agent. Any stress noted to the fetus will be conveyed to the veterinarian.

Blood sampling: A potential problem is increased ketamine tolerance, anemia/low hematocrit and sensitivity at injection site. Ketamine tolerance will be managed by using the lowest dose possible for the procedure. We will monitor hematocrit by taking a blood gas reading bi-weekly from animals on study. Vitamin supplements will be given. If prolonged anemia is seen, the Veterinarian will be consulted. To reduce sensitivity at the injection site, when possible the animal will be injected at different sites on the rump or large leg muscle area. In addition to ensure that animals health is not compromised, blood draw will not exceed 10% of the circulating blood volume over a 2-week period. Also note that total blood volume collected from each animal will not exceed 12mL/kg/month

14b. Describe the clinical parameters that will be monitored to indicate adverse effects, pain, and/or distress to animals. The parameters should be specific to the species and to the procedure(s). Include the frequency of monitoring throughout the study...

Animals in the baboon colony are checked daily by PI staff and twice daily by CompMed staff. All staff will determine if each animal is eating, urinating, passing stool, and demonstrating the repertoire of behaviors normal for the individual animal. Immediately after surgery and during the postoperative period as defined by IACUC policy, animals will be observed daily by PI staff, with these observations recorded on postoperative evaluation sheets which become part of the animals' permanent records. Postoperative evaluation will include specific assessment of pain; failure to eat or decreased appetite, drink, urination or fecal output, change in normal repertoire of behaviors, may indicate pain. Lethargy and guarding of the incision site(s) may also indicate pain. If any of the above is seen during the postoperative monitoring period, the Veterinarian will be notified.

14c. What conditions and/or complications would lead to removal of an animal from the study (*i.e.an early endpoint*)?

Spontaneous abortion is cause for removal from protocol study. Also, as noted previously, if an animal is receiving Letrozole injections and suffers a seizure, the animal will be removed from that treatment group. Animals will be considered for euthanasia as described in the IACUC protocol entitled "Guidelines for Early Removal Criteria and the Use of Death as an Experimental Endpoint". In addition, the Veterinarian may remove an animal from a protocol if a significant health problem is identified.

G. ADMINISTRATION OF ANESTHESIA, THERAPEUTICS AND EXPERIMENTAL AGENTS

15. Indicate the sedatives/tranquilizers, anesthetics, analgesics, antibiotics, and other relief agents that will be administered. If no anesthetics, analgesics, or other pain relief methods will be used, please provide a strong justification for withholding analgesic agents in Question 15a below. The withholding of analgesic agents must be based upon cited scientific fact or provided experimental data. NOTE: **Some anesthetics and analgesics are controlled substances and require Virginia Board of Pharmacy and DEA licenses for purchase and use.** ADD ADDITIONAL ROWS AS NEEDED...

	Dose (mg/kg)	Route	Frequency	
Sedatives/Tranquilizers				
Anesthetics – General				
ketamine-HCl	5-10 mg/kg	IM	Chemical restraint for blood sampling (1-4 days a week), sedation for IVGTT, ultrasound exam, or preoperatively	
ketamine-HCl in 250ml 0.9% Sodium Chloride IV bag	5-10 mg/kg	IV	Chemical restraint for IVGTT, once every 6 months	
Isoflurane gas	MAC% is average ~2% for maintenance	Inhaled	At surgery, fetal injection, and during amniocentesis for isolation of amniotic fluid) for determination of fetal sex.	
Anesthetics – Local				
Bupivacaine	0.5%	Topical	Once at surgery prior to closing skin layer	
Analgesics			**Frequency**	**Length of Administration**
Buprenorphine	0.03 mg/kg	IM	At surgery and 6-8hours postoperatively	3 days BID followed PRN up to 5 days
Ketoprofen	2 mg/kg	IM/oral	SIB 3days	at surgery and day 2 and 3 post-operatively
Antibiotics				
Penicillin and streptomycin	100 U/ml penicillin and 100 µg/ml streptomycin	IV	At insertion of cannula/mini-pumps. Antibiotics in saline and incorporated into the 2ML2 and 2001D mini-pumps to deliver vehicle at 5 µl/hr and 8 µl/hr, respectively or total volume of 312 µl/day or 30U/ 30 µg Pen/Strep/day.	Throughout the 20 day experimental period (see gonado-tropin challenge test)
Miscellaneous				
glycopyrrolate	0.005-0.01 mg/kg	IM	one dose at induction of anesthesia	one dose
intravenous fluids: 0.9% sodium chloride or similar solution for IV administration	approximately 10 ml per kg body weight per hour during surgery;	IV	one dose intraoperative	one dose intraoperative
Iron Dextran	10 mg/kg, IM	IM	At completion of surgery, then PRN not to exceed Q 7days	

15a. **JUSTIFICATION FOR WITHHOLDING ANALGESIC AGENTS**

Analgesics will not be withheld.

16. Will agents other than anesthetics or analgesics (*i.e. drugs, reagents, cells, etc*) be administered?

X YES (*Complete **Question 17** for each agent. Add additional sections as needed.*) ____ NO (*Skip to **Question 18***)

17. Agent: Letrozole Agent vehicle: Sesame oil (sterile)
Route/site: SQ to the mother Volume per administration: 0.2 to 1.0 ml
Frequency of administration: Daily on days 100 to 169 or on days 100-110 of gestation
Expected side effects and/or changes in the animal's behavior:
Depreciated appetite. Although the drug itself does not elicit any side effects, the fact that the consequence of drug therapy is a decrease in estrogen production/levels by >95%, we observe in 15% -20% of pregnancies premature delivery and/or maternal seizures. In instances where a mild seizure has occurred but animal has not become comatose, we stop drug treatment for 24-48 hours. Drug treatment can then be re-initiated without further development of any problems. See section 14a.

17 Agent: Ovine Prolactin Agent vehicle: Sterile saline (0.9%)
Route/site: SQ to the mother Volume per administration: 1.0 ml
Frequency of administration: Daily starting five days before anticipated cesarean section in animals treated on days 100-165 of gestation with Letrozole to facilitate milk production. Estrogen-suppressed animals (i.e. treated with Letrozole alone) do not synthesize prolactin which is required for milk production and thus breast feeding of newly delivered fetus/neonate is compromised.
Expected side effects and/or changes in the animal's behavior:
None anticipated. It is possible that prolactin may induce spontaneous delivery although this is not an adverse event and has not been seen in our study. It is also possible that milk production will not be sufficiently restored.

17. Agent: Estradiol 17β- 3 benzoate Agent vehicle: Sesame oil (sterile)
Route/site: SC to the mother Volume per administration: 0.2 to 1.0 ml
Frequency of administration: Administered daily on days 100-169 of gestation in conjunction with Letrozole to restore estrogen production. Also administered daily on days 25-59 of gestation in otherwise untreated baboons to prematurely elevate estrogen levels in early gestation. Finally, also administered on days 108 and in conjunction with Letrozole to restore estrogen production in short-term estrogen-suppressed animals.
Expected side effects and/or changes in the animal's behavior: None anticipated

17. Agent: Estradiol 17β Agent vehicle: Saline/10% ethanol
Route/site: Maternal saphenous IV Volume per administration: < 0.2 ml
Frequency of administration: Once as a bolus to rapidly increase estrogen levels (e.g. 2h and 6h) on day 54 or day 110 of gestation in untreated animals (day 54) or in animals being treated on days 100-110 or 120-140 with Letrozole.
Expected side effects and/or changes in the animal's behavior: None are anticipated.

17. Agent: Human Luteinizing Hormone (hLH) Agent vehicle: Sterile saline with 100U penicillin/ml and 100 µg/ml streptomycin Sesame oil
Route/site: 2ML2 ALZET Osmotic Minipump attached to an indwelling intra-venous cannula Volume per administration: 5 µl/hr or 120 µl/day x 10 days = 1.2 ml; dose of LH to be delivered = 4.8 µg/kg BW/day
Frequency of administration: Pump in place for 10 days; to deliver 4.8 µg hLH/day/kg BW
Expected side effects and/or changes in the animal's behavior: None anticipated

17. Agent: Human Follicle Stimulating Hormone (hFSH) Agent vehicle: Sterile saline with 100U penicillin/ml and 100 µg/ml streptomycin
Route/site: 2001D ALZET Osmotic Minipump attached to an indwelling intravenous cannula Volume per administration: 8 µl/hr or 192 µl/day; dose = 480-720ng FSH/day/kg BW; replace pump daily and increase dosage at 3-day intervals, i.e. 480 ng/kg BW days 1-3; 600 ng FSH/day/kg BW days 4-6 and 720 ng FSH/day/kg BW days 7-9
Frequency of administration: FSH pump changed daily (LH pump not changed)
Expected side effects and/or changes in the animal's behavior: None anticipated

17. Agent: Leuprolide (GnRH antagonist) Agent vehicle: Sterile Saline
Route/site: SC Volume per administration: < 0.5 ml
Frequency of administration: Once every other day as a bolus to shut down endogenous production of LH and FSH by the pituitary gland
Expected side effects and/or changes in the animal's behavior: None anticipated

17 Agent: [1,2]-^{3}H cortisone Agent vehicle: Sterile Dextrose/water
Route/site: IV infusion to mother/young adults Volume per administration: 0.4ml/min x 90 min
Frequency of administration: Once during surgery on day 160-170 of gestation in baboons untreated or treated with Letrozole with/without estradiol benzoate. This study is also performed in young baboon neonates at age 60 months under Ketamine/IV drip
Expected side effects and/or changes in the animal's behavior: None

17. Agent: [4]-^{14}C cortisol Agent vehicle: Sterile Dextrose/water
Route/site: IV infusion to mother/young adults Volume per administration: 0.4 ml/min x 90 min
Frequency of administration: Once during surgery on day 170 of gestation in baboons untreated or treated with Letrozole with/without estradiol benzoate. This study is also performed in young baboon neonates at age 60 months under Ketamine/IV drip
Expected side effects and/or changes in the animal's behavior: None

17. Agent: [1,2] ^{3}H dehydroepiandrosterone Agent vehicle: Sterile Dextrose/water
Route/site: IV infusion to young adult Volume per administration: 0.4 ml/min x 90 min
Frequency of administration: This study is performed in young baboon neonates at age 60 months under Ketamine/IV drip
Expected side effects and/or changes in the animal's behavior: None

17. Agent: [4]^{14}C dehydroepiandrosterone sulfate Agent vehicle: Sterile Dextrose/water
Route/site: IV infusion to young adult Volume per administration: 0.4 ml/min x 90 min
Frequency of administration: This study is performed in young baboon neonates at age 60-82 months.
Expected side effects and/or changes in the animal's behavior: None

17. Agent: Estradiol-17β Agent vehicle: Oil
Route/site: IM to fetus Volume per administration: 1µg / 0.2ml sesame oil
Frequency of administration: Once at time one hour of the experiment on d140 or 165 of gestation.
List all expected side effects and/or changes in the animal's behavior: There are no expected changes in animal behavior from this agent. Possible side effects include: irritation at injection site

17. Agent: U0126 Agent vehicle: 1% DMSO
Route/site: IM to fetus Volume per administration: 100mg/kg BW/ 0.5 ml
Frequency of administration: Once at time "0" of the experiment on D165 of gestation
List all expected side effects and/or changes in the animal's behavior: There are no expected changes in animal behavior from this agent. Possible side effects include: irritation at injection site

17. Agent: Wortmannin Agent vehicle: 1% DMSO
Route/site: IM to fetus Volume per administration: 1.4 mg/kg BW/0.5 ml
Frequency of administration: Once at time "0" of the experiment on D165 of gestation

List all expected side effects and/or changes in the animal's behavior:
There are no expected changes in animal behavior from this agent. Possible side effects include: irritation at injection site

17. Agent: 1% DMSO (vehicle control) Agent vehicle: 1% DMSO

Route/site: IM to fetus Volume per administration: 0.5 ml

Frequency of administration: Once at time "0" of the experiment on D165 of gestation
List all expected side effects and/or changes in the animal's behavior:
There are no expected changes in animal behavior from this agent. Possible side effects include: irritation at injection site

17. Agent: Neomycin Agent vehicle: *Saline*

Route/site: IM to fetus guided via ultrasound Volume per administration: *10mg-50mg/kg BW*

Frequency of administration: Once at time "0" of the experiment on D165 of gestation
List all expected side effects and/or changes in the animal's behavior:
There are no expected changes in animal behavior from this agent. Possible side effects include: irritation at injection site, nephrotoxicity

Agent: Y27632 Agent vehicle: 1% DMSO

Route/site: IM to fetus Volume per administration: 1.4 mg/kg BW/ 0.5 ml

Frequency of administration: Once at time "0" of the experiment on D165 of gestation
List all expected side effects and/or changes in the animal's behavior:
There are no expected changes in animal behavior from this agent. Possible side effects include: irritation at injection site

NOTE: Your signature on page 4 certifies that all drugs used on animals before, during, or after an experimental or surgical procedure will be obtained from legal sources, will be pharmaceutical-grade, unless otherwise approved, and will be disposed of properly when out-of-date or no longer needed. **All controlled substances MUST be kept in a double-locked compartment, and records documenting each use of a controlled substance MUST be maintained.**

H. SPECIES SELECTION AND ORDERING:

18. Please indicate the species and number of animals requested:

Species (Common Name & Strain)	Total Number Requested for a 3 Year Period	Avg. # to be Maintained in Animal Facility	Max. # to be Maintained in Animal Facility
Baboon (*Papio anubis/cynocephalus*)	40	29	47

If the project involves NON-HUMAN PRIMATES,
Complete Attachment B: NON-HUMAN PRIMATE ENHANCEMENT PROCEDURE.

19. Will animals be ordered through the Division of Comparative Medicine?

X YES _____ NO (***Identify source and provide the rationale/justification)***

20. Will the animals require special housing (e.g., specific bedding requirements, isolator cages, special feed or handling, etc?)

X YES (***Describe all special requirements***) _____ NO

Animals utilized for the Isotope study will require special handling and disposal of feces and urine. EVMS Radiation Safety Manual section 13 and 14 outline radioactive waste disposal in animal studies and animal use procedures. PI staff along with CompMed staff will follow these guidelines when animals are on study. Radiation Safety officer will be notified prior to starting this portion of the protocol in order to coordinate proper handling and disposing of the waste products.

21. Does this study involve the use of animals that will be maintained as a colony over a long period of time? (**Colony is defined as "breeding or holding of animals for reuse in other experiments."**)

X YES (*Complete **Questions 21a and 21b***) _____ NO (*Skip to Question 22*)

21a. List the number of new animals you are planning to purchase for the colony: 9

21b. List the number of animals you are planning to use from an existing colony: 20

I. PERSONNEL TRAINING:

22.	In Section 1, list the name of each person involved with the project, along with the species to be used, the person's years of experience with that species, and the person's training information. In Section 2, continuing with the column from Section 1, note each person's functional role for each species listed. **ADD COLUMNS OR PAGES AS NEEDED.**

SECTION 1: PERSONNEL INFORMATION						
NAME:	Gerald Pepe	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
(Species / Experience)	**Species / Exp**	**Species / Exp**	**Species / Exp**	**Species / Exp**	**Species / Exp**	**Species / Exp**
Species used in project / Years of Experience with each species listed	P anubis/ 33	P anubis/12	P. anubis/9	P. anubis/9	P. anubis/ 2	P. anubis/ 2
	/	/	/	/	/	/
	/	/	/	/	/	/
Occupational Health and Safety (OHSP) Training Certificate Number:	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Occupational Health and Safety Risk/Health Assessment Date (Month / Year)	7/24/00	7/24/00	3/8/04	9/12/00	9/10/01	6/14/05
LATA Training by species:	Nonhuman primate	Nonhuman primate	Nonhuman primate/Rodent(mouse)	Nonhuman primate	Nonhuman primate	Mouse and primate
SECTION 2: FUNCTIONAL ROLE FOR EACH SPECIES LISTED						
Supervision	X		X	X		
Care and Handing	X	X	X	X	X	
Anesthesia	X		X	X		
Surgery	X	X	X	X	X	X
Post-Surgical Care			X	X	X	
Monitoring	X	X	X	X	X	
Euthanasia	X		X	X		

NAME:	[redacted]					
(Species / Experience)	Species / Exp	Species / Exp	Species / Exp	Species / Exp	Species / Exp	Species /Exp
Species used In project / Years of Experience with each species listed	M. mulatta /15	/	/	/	/	/
	/	/	/	/	/	/
	/	/	/	/	/	/
Occupational Health and Safety Training Cert. No.:	[redacted]					
Occupational Health and Safety Health Assessment (Month / Year)	3/6/07					
LATA Training by species:	Nonhuman primate					

SECTION 2: FUNCTIONAL ROLE FOR EACH SPECIES LISTED:						
Supervision						
Care and Handling						
Anesthesia						
Surgery	X					
Post-Surgical Care						
Monitoring						
Euthanasia						

22a. Please provide information regarding the degree of training and procedural experience for <u>each</u> individual listed in Questions 22.

Dr. Pepe has performed surgeries on baboons for more than 30 years. His team works closely with him to provide for collection of tissues during surgery. [redacted] and [redacted] have had a wealth of experience working with these animals and are expert in intubation/surgical preparation and surgical assistance. Moreover, [redacted] has been trained by Dr. Pepe and has been performing surgeries (cesarean section) without direct assistance of Dr. Pepe (e.g. Dr. Pepe has not scrubbed in but is available or on site) for the past two years. [redacted] has had significant experience assisting Dr. Pepe in the conduct of the surgical experimentation and to collect tissue samples. [redacted] has had over 7 years of experience with surgery in nonhuman primates and is available to assist should [redacted] or others be sick or unavailable. [redacted] is a graduate student and will not be performing surgery; she is there to assist in record-keeping, weighing fetal tissues as they are collected and of course to become familiar with and thus learn (i.e. see and observe). [redacted] has had several years experience working with nonhuman primates including rhesus monkeys and has experience with and known for his expertise in performing placement of endometrium cannulas/Alzet mini pumps to examine ovarian function.. None of these need be listed as technical coordinator or co-investigator. A lot of tissues are collected and these individuals as well as others listed are required to provide

the help to accomplish this task in a timely manner.

22b. List any person that will require supplemental training from the Division of Comparative Medicine and describe the desired training.

None required

J. ANIMAL USE PROCEDURES (EXCEPT SURGICAL PROCEDURES):

ALL SURGICAL PROCEDURES MUST BE DETAILED IN *ATTACHMENT E, ANIMAL SURGICAL PROCEDURES*

23. Will cells, tissues and organs be collected?

X YES (*Complete all applicable procedures below)* _____ NO (*Skip to* ***Question 24***)

23a. **Blood sampling**:

Technique: **[1] Pregnancy studies**: Blood samples (maternal) are collected at 1-4 day intervals between days 25-60, 100-110 or 100-175 of gestation (term = d184). Briefly, animals are restrained, injected with ketamine-HCl (10 mg/kg) and samples (3-5 ml) obtained from a saphenous or antecubital vein using a 21 gauge needle. **[2] Neonate-Adolescent studies**: Blood samples are obtained once every two weeks from neonates and prepubertal adolescents. Briefly, baboons are restrained, injected with ketamine HCl (10 mg/kg BW) and a 2 ml (neonates) sample obtained from a peripheral saphenous vein using a 21g needle. Weight is also recorded and monitored in this group.**[3] IVGTT Studies**: At age 12, 24, 30, 36, 42, 48, 54, and 60 months, adolescents will undergo intravenous glucose tolerance testing (IVGTT). Samples are collected into heparinized syringes via catheter at 0, 1, 3, 5, 10, 20, 40, 60 and 90 mins and 0.1 ml examined for blood glucose and the remainder kept on ice and serum subsequently assayed for insulin/C-peptide. **[4] Ovarian function studies**: To determine ovarian function, blood samples will be obtained from these animals daily during two menstrual cycles (cycle one: blood samples obtained; cycle two: no sampling; cycle three: blood samples obtained). Briefly, adolescent baboons (10-14 kg BW) are injected with ketamine HCl (10 mg/kg BW), and a 2-3 ml sample obtained from a peripheral saphenous vein using a 21g needle. **[5] Gonadotropin Challenge studies:** At approximately 90-96 months of age, blood samples (3-5 ml) will be obtained from baboons over a 10-day period following insertion into the right jugular vein of indwelling cannulas to which are attached Osmotic minipumps designed to deliver pituitary hormones LH or FSH.

Sample site: Saphenous/antecubital/femoral;

Volume per sample: 3-5 ml from adults; approximately 1 ml from neonates/adolescents until puberty (e.g. age 4 yrs); approximately 3.0ml over a 90 min period (IVGTT) in these neonates at 12, 24, 30, 36, 42, 48, 54 and 60 months of age; 2-3 ml from regularly menstruating female adolescents (i.e. same animals now >60 months of age); not to exceed 10% of circulating blood volume over a 2-week period. Blood draw will not exceed 10% of the circulating blood volume over a 2-week period. Also note that total blood volume collected from each animal will not exceed 8 ml/kg/month.

Frequency & duration of sampling: 1-4 day intervals during gestation from mother; once every other week from adolescents; at 6 to 12 month intervals for IVGTT; daily during 2 non-consecutive menstrual cycles in adolescents exhibiting regular menstrual cycles and daily over a ten day period in animals 90-96 months of age.

23b. Urine/feces sampling:

In studies using isotope dilution/constant infusion procedures to study hormone production in adolescent animals and uptake/transfer across the placenta in pregnancy, urine voided into and fecal material present in pans beneath animal cages is collected during the immediate 6h period and the subsequent 24h, 48h and 72h. A sample (0.5 ml) is tested for radioactivity and specimens made available to Radiation Safety office for consideration of disposal route

Method: Approximately 95% of injected isotope is eliminated via urine within 48h.

Frequency & duration of sampling: 6h, 24h 48h and 72 h after onset of infusion.

23c. **Collection of tissues:**

Tissues collected: Kidney, liver, lung, gonads, adrenal, pituitary, pancreas, skeletal muscle, visceral and SQ fat, intestine and uterine samples.

When collected (*before or after euthanasia*): After euthanasia in both adult sacrificed animals and fetus following euthanasia at surgery

Disposition of collected tissues: Fixed and/or frozen for experimentation.

24. Will behavioral testing be conducted?

X No behavioral testing will be conducted

_____ Yes, behavioral testing will be conducted with significant restraint or noxious stimuli. (*Complete **Attachment C, PROLONGED PHYSICAL RESTRAINT OR STRESS***)

_____ Yes, behavioral testing will be conducted without significant restraint or noxious stimuli. (***Describe the procedure below.***)

25. Will a special diet be required?

X YES (*Complete the **Questions 25a-25c***) _____ NO (*Skip to **Question 26***)

25a. Describe the anticipated nutritional deficit or supplementation:
Vitamins with iron

25b. Provide the reason(s) for and treatment of the deficit or supplementation:
To prevent anemia in animals undergoing blood sampling during pregnancy, IVGTT and or during normal menstrual cycles

25c. How often will animals be weighed?
Adolescents are weighed bi-weekly; pregnant animals, before initiation of experimental drug treatments and at least once during the treatment.

How much weight change will be permitted before the animal will be removed from the study? 20-25%

26. Will indwelling catheters or implants be used?

X YES (*Complete a section below for each site. Add additional sections as needed.*) _____ NO (*Skip to **Question 27***)

26a(1) Site: Cannula (Becton-Dickinson) inserted into right jugular vein and tunneled to the subscapular region and attached to Alzet osmotic minipumps which are inserted under the skin. Placement into the jugular veins permits use of a small cannula that that required if inserted into the femoral veins.

Type & Size: 2ML1 osmotic pump (5.1 cm length x 1.4 cm diameter; 5.1 grams weight) with a 3.5 cm 25 gauge tube and a pumping rate of 10 µl/hr; 2001D osmotic pump (3.0 cm length x 0.7 cm diameter; 1.1 grams weight) with a 2.2 cm 27 gauge tube and a pumping rate of 8 µl/hr

Maintenance: Osmotic minipump infusion of saline or hormone FSH or LH

Duration: 20 days (during first 10 days pumps deliver saline/antibiotic vehicle only). FSH/LH initiated on day 10

26a(2) Site: _____

Type & Size: _____

Maintenance: _____ Duration: _____

27. Will tumors be transplanted or induced?

_____ YES *(Complete a section below for each site. Add on more sections as needed.)* X NO *(Skip to **Question 28**)*

27a(1) Transplant or Induction Site: _____

Anticipated Functional Deficit(s) and Management: _____

Maintenance: _____ Duration: _____

27a(2) Transplant or Induction Site: _____

Anticipated Functional Deficit(s) and Management: _____

Maintenance: _____ Duration: _____

K. ANIMAL CARE:

28. Describe, in detail, the plans for medical care of the animals in the proposed study, and **identify, by name and job classification**, the responsible person(s) on the investigative staff. **NOTE: *Routine observation of the animals and medical intervention is the responsibility of the principal investigator.***

All animals will be observed daily by [redacted], Animal Coordinator/Research Associate, and/or [redacted], both trained members of the PI's (Gerald J. Pepe) staff. Medical problems will be reported to the Veterinarian or a member of the CompMed staff. Postoperative monitoring will be performed for each animal after surgery as defined by IACUC policy. The animal's attitude (alert, responsive) is observed as well as the status of the surgical incision(s), food consumption, urine and feces production, and resumption of the animal's normal repertoire of behaviors; pain is also assessed as described. Postoperative observations are recorded on forms approved by CompMed, and these forms become part of the animal's permanent record.

29. Will this study require special observation?

X YES *(Complete **Question 29a**)* _____ NO *(Skip to **Question 30**)*

29a. Frequency of Observation: daily

By whom *(Identify by Name)*: _____

[redacted] will be primarily responsible for all observations described above. Animals will also be observed daily to determine if menstruations have occurred. The PI or a trained member of the PI's staff will observe animals in [redacted] absence.

Starting: daily Ending: daily

30. Indicate any special instructions that should be taken for animals found dead (e.g., call Investigator, refrigerate or freeze carcass, disposal, etc.). If you would like to have the Institutional Veterinarian necropsy animals that die unexpectedly, please indicate how you would like the tissues to be handled: Indicate any special instructions that should be observed for animals found dead (*e.g., call investigator, refrigerate or freeze carcass, disposal, etc.*). **If you would like for the Institutional Veterinarian to necropsy animals that die unexpectedly, please indicate how you would like for the tissues to be handled.**

Alert the investigator and for emergency animal care contact [redacted]. If necropsy is performed, collect uterus and adrenals, and pieces of kidney and liver (or others as determined by the Veterinarian) which are placed in fixative (4% paraformaldehyde or phosphate buffered formalin) for subsequent histopathology.

L. DISPOSITION OF ANIMALS:

31. Please indicate the means of animal disposition (**Check all that apply**):

X Euthanasia *(Complete **Questions 33a-33c**)*

____ Death as an Endpoint *(Complete **Attachment G: DEATH AS AN ENDPOINT**)*

____ Return to animal colony

X Available for transfer into another IACUC protocol *

X Available for transfer to another research institution*

____ Available for adoption as a companion

____ May be culled for tissue sharing

____ Other (Explain):

* ***Animals that have undergone survival surgery in one IACUC protocol may not be transferred to another survival surgical protocol, unless the request is specifically reviewed and approved by the IACUC. These animals may be transferred to non-surgical or non-survival protocols without IACUC review.***

32. Disposition of Surviving Animals

32a. Will animals survive the protocol/procedures?

X YES *(Complete **Question 32b**)* ____ NO *(Skip to **Question 33**)*

32b. Will animals survive without harm or disability?

X YES *(Skip to **Question 33**)* ____ NO *(Complete **Question 32c**)*

32c. Describe the harm or disability and the plans for management of the disability.

33. Euthanasia

33a. Will the animals be euthanized?

X YES *(Complete **Questions 33b-33d**)* X NO *(Skip to **Question 34**)*

33b. Explain why the animals will be euthanized:

The maximal number of multiple survival surgeries has been achieved or the animal has developed problems either protocol related or clinical which compromise further surgical interventions. We do attempt to relocate these animals and/or employ them as surrogate mothers for developing neonates. In addition, there is need to collect adult tissues (ovaries, adrenal etc) to serve as controls for our fetal and adolescent studies. In a small % of the colony, we are unable to achieve a pregnancy for reasons not clear to us (the animal does not have normal menstrual cycles, is not mensing at all or is not receptive to breeding). In this instance, we request that these few (<5%) animals be transferred to University of Maryland to be used in our study there.

33c. Indicate how the animals will be euthanized:

Euthanasia agent/procedure: Pentobarbital Solution (390mg/ml)

Dose: 1 ml/4.5 kg body weight Route: IV

33d. Per the *AVMA (American Veterinary Medical Association) Guidelines on Euthanasia*, June 2007 (formerly the *2000 Report of the AVMA Panel on Euthanasia*), most physical methods of euthanasia, when done appropriately, are "conditionally acceptable," meaning that the nature of the techniques may not consistently produce humane death or they present a greater potential for operator error or safety hazards. In those situations where physical methods may be the most appropriate method for euthanasia and rapid relief of pain and suffering, extreme care and caution must be exercised, and personnel performing physical methods of euthanasia must be well trained and monitored for each type of physical technique. **If a physical method, such as decapitation or cervical dislocation, will be used as the primary means of euthanasia, please provide scientific justification.**

M. ANIMALS BROUGHT INTO AND TAKEN OUTSIDE OF THE ANIMAL FACILITY:

34. Will any animals be transferred into the EVMS Animal Facility from another institution?

X YES *(Complete **Questions 34a-34b**)* _____ NO *(Skip to **Question 35**)*

All animals received from other than approved vendors must undergo a quarantine period to allow for evaluation of the health status of the animals prior to their introduction into the colony. They may also require testing and segregation to determine their health status.

THE PRINCIPAL INVESTIGATOR SHOULD DISCUSS THESE ISSUES WITH THE DIVISION OF COMPARATIVE MEDICINE PRIOR TO INITIATING ANIMAL TRANSFER. THE PRINCIPAL INVESTIGATOR IS RESPONSIBLE FOR ALL RELATED CHARGES.

34a. How long will the quarantine or stabilization period last?

Three completed negative TB test results or as determined by the Institutional Veterinarian

34b. How long will the animals be housed at EVMS?

Animals will be housed at EVMS until 1) the quarantine period is completed, 2) the assigned protocols are completed, and 3) reassignment within EVMS, transfer out of EVMS, or euthanasia. Generally, animals complete the assigned protocols within 1 year. Quarantine and preparation for disposition will add approximately 6 months. Unforeseen problems which disrupt the group may require additional months for stabilization of menstrual cycles before experiments can begin or continue.

35. Will the animals be taken out of the Central Animal Facility (*i.e. CompMed, [redacted] floor*) for any reason (*i.e. manipulation, surgery, temporary housing, etc.*)?

_____ YES *(Complete **Questions 35a-35c**)* X NO *(Skip to **Question 36**)*

35a. To what building(s) and room(s) will the animals be taken? *(Indicate what procedure(s) will be performed in each specific location.)*

35b. How will the animals be transported? (*Be specific. Include all safety precautions for animals and personnel.*)

35c. How often will the animals be taken to the location(s) listed above, and for what duration of time per incident?

36. Will the animals be used or housed in locations outside of the Central Animal Facility (*i.e. CompMed, [redacted] floor*) for periods greater than 12 hours?

_____ YES *(Complete **Questions 36a-36c**)** X NO

***The location must be certified as a satellite-care facility and undergo semi-annual inspection by the IACUC.**

37a. In what building(s) and room(s) will the animals be used or housed?

37b. Describe the animal husbandry to be performed, and identify, by name, the person(s) who will provide husbandry.

37c. How long will the animals be used or housed in the satellite-care facility?

Eastern Virginia Medical School
Institutional Animal Care and Use Committee

Attachment B: Nonhuman Primate Enhancement Procedure

Project Title: Regulation of Fetal-Placental Development in the Primate

1. Paired housing: Nonhuman primates used under this protocol can be housed in the same primary enclosure with one or more compatible primates.

   X YES (*Skip to Question 2*) _____ NO (*Complete Question 1a*)

   1a. Justify why the animal must be singly housed:

   The PI supports social housing of research primates. However, housing must allow PI/PI staff access to individual animals at all times for blood sampling, injection, and accurate postoperative evaluation.

2. Nonhuman primates used under this protocol will be provided with a variety of devices as described in the EVMS Primate Enhancement Program (this can be provided to you by the Office of Research or the Division of Comparative Medicine (CompMed) upon request).

   X YES _____ NO (*justify in the space below*):

Eastern Virginia Medical School
Institutional Animal Care and Use Committee

Attachment D: Use of Hazardous Agents

Project Title: Regulation of Fetal-Placental Development in the Primate

This form applies to IACUC protocols using materials such as radioisotopes, infectious agents, carcinogens, biohazards, mutagens, teratogens, abortifacients, acutely toxic chemicals or other potential chemical hazards. Information provided in this form will enable assessment of risk to individuals handling or caring for animals, as well as risk to other animals. If you need assistance responding to any of the questions, please contact the Environmental Health and Safety Office at (757) [redacted]

YOU MUST COMPLETE A SEPARATE FORM FOR EACH HAZARDOUS AGENT USED.

1. Please indicate the types of hazardous agent being utilized. Check all that apply. For microorganisms, please list the Risk Group or Biosafety Level. For chemicals, list the CAS and the LD50 from the Material Safety Data Sheet (MSDS).

   X Radioactive material (Isotope: ^{14}C)
   ____ Infectious agent (Risk Group or Biosafety Level: _____)
   ____ Acutely toxic chemical (CAS: _____, LD50: _____)
   ____ Known or suspected human carcinogen (Chemical Name: _____)
   ____ Known or suspected human mutagen/teratogen (Chemical Name: _____)
   ____ Recombinant DNA/RNA
   ____ Tissue sample (Type: _____)
   ____ Other (*please describe below*):

2. Please provide specific information about the agent:

   Complete name *(include strain for microorganisms)*: ^{14}C labeled steroid hormone
   Dose and frequency: IV infusion; one for 90 minutes Concentration: ≤ 20μCi/animal
   Route: IV Duration of exposure: 96hrs
   How long is the animal maintained after administration? 6 months to 3years
   Animal species: Baboon Estimated animal weight: 15kg

3. Is the agent excreted or shed by the animal?

X YES *(Please indicate the type of excreta and estimated quantity per day)* _____ NO

90% of injected dose excreted into urine within 48hours; 10% by feces within 48 hours. Excreted as intact hormone – not as water; not as CO_2

4. Are there documented human risks from exposure to the agent (risks may be determined from the MSDS or from references contained in the Biosafety Manual):

X YES *(Complete Questions 4a-4e)* _____ NO *(Skip to Question 5)*

4a. Indicate the route of human exposure:

_____ Inhalation	X Contact
X Ingestion	_____ Parenteral
_____ Other (describe below):	

4b. Describe the risks associated with the agent (mutagen, teratogen, etc.):

Doses are extremely low thereby limiting risks significantly

4c. Indicate the acutely toxic/infectious dose in humans (in mg/kg or number of organisms):

The ALI for carbon 14 is 2mCi; this allowable level of ingestion is greatly in excess of the dose being injected which is approximately 0.02mCi total. Therefore should be no toxic/infectious dose.

4d. Describe any genetic changes to the organism and their suspected effects:

None

4e. Describe the symptoms of exposure:

Nausea and vomiting are usually the initial symptoms of a mile exposure. Radiation poisoning if person should ingest more than 2mCi of ^{14}C

4f. Describe the first aid methods to be taken in the case of exposure:

Radiation safety will immediately be contacted and EVMS guidelines for exposure as outlined in Appendix A Emergency Procedures will be followed. See attached to protocol or EVMS website

4g. Indicate all personal protection required:

X Lab coat/dedicated clothing	_____ Apron
X Gloves	X Face shield
X Goggles	X Respirator
_____ Other (Describe below):	

5. Are there risks to other animals in the room or in the animal facility?

X YES *(Complete Questions 5a-5e)* _____ NO *(Skip to Question 6)*

5a. Describe the risk to other animals:

Exposure to radioactive material (minimal to none)

5b. Indicate the route of animal exposure:

Ingestion

5c. Describe all methods that will be used to contain the risk factor:

Animals under isotope study will be confined to single housing with no tactile contact with other animals until isotope has cleared animals system (usually within 72 hours) and exposure is eliminated.

5d. Are special animal care requirements necessary?

_X___ YES *(Describe below)* _____ NO

Please see EVMS Radiation Safety manual section 14. Urine and feces will be collected until isotope has cleared the animals system. Animal/s under study cannot be pair housed or close to tactile contact with another animal. Animal does NOT have to be quarantined or isolated.

5e. Are special waste or carcass disposal requirements necessary?

_X___ YES *(Describe below)* _____ NO

Please see EVMS Radiation Safety manual section 13.2 and section 14

6. Briefly describe the procedure(s) for administration of the agent and how the agent is metabolized and/or excreted. Include how long the agent is expected to remain in/be shed from the animal, concentration of agent in any specific organs, etc.

Radioactive hormones are administered IV via a maternal saphenous vein for 60minutes at ≈ dosage of 10μCi ^{14}C steroid (total 20μCi) total. Blood samples which are collected at 0, 50, 55, and 60 minutes are used to determine clearance rates and interconversion of ^{14}C isotope labeled hormones (cortisol, cortisone, testosterone, androstenedione) Steroid hormones (^{14}C labeled as well) are excreted without further breakdown and because of locations of ^{14}C in the molecule, no water or $^{14}CO_2$ is formed. Steroids are cleared from the animal via urine (90%) and feces (10%) within 48-72hours.

6a. Have all laboratory personnel and CompMed staff been instructed of these hazards and received training on proper handling techniques?

_X___ Laboratory Personnel _X___ CompMed Staff

7. Please provide any other relevant information regarding the agent (e.g., unique containment recommendations): N/A

Eastern Virginia Medical School
Institutional Animal Care and Use Committee

Attachment D: Use of Hazardous Agents

Project Title: Regulation of Fetal-Placental Development in the Primate

This form applies to IACUC protocols using materials such as radioisotopes, infectious agents, carcinogens, biohazards, mutagens, teratogens, abortifacients, acutely toxic chemicals or other potential chemical hazards. Information provided in this form will enable assessment of risk to individuals handling or caring for animals, as well as risk to other animals. If you need assistance responding to any of the questions, please contact the Environmental Health and Safety Office at (757) [redacted]

YOU MUST COMPLETE A SEPARATE FORM FOR EACH HAZARDOUS AGENT USED.

1. Please indicate the types of hazardous agent being utilized. Check all that apply. For microorganisms, please list the Risk Group or Biosafety Level. For chemicals, list the CAS and the LD50 from the Material Safety Data Sheet (MSDS).

- X Radioactive material (Isotope: ^{3}H)
- ____ Infectious agent (Risk Group or Biosafety Level: _____)
- ____ Acutely toxic chemical (CAS: _____, LD50: _____)
- ____ Known or suspected human carcinogen (Chemical Name: _____)
- ____ Known or suspected human mutagen/teratogen (Chemical Name: _____)
- ____ Recombinant DNA/RNA
- ____ Tissue sample (Type: _____)
- ____ Other (*please describe below*):

2. Please provide specific information about the agent:

Complete name
(include strain for microorganisms): ^{3}H labeled steroid hormone

Dose and frequency: IV infusion; one for 90 minutes Concentration: ≤ 20μCi/animal

Route: IV Duration of exposure: 96hrs

How long is the animal maintained after administration? 6 months to 3years

Animal species: Baboon Estimated animal weight: 15kg

3. Is the agent excreted or shed by the animal?

X YES *(Please indicate the type of excreta and estimated quantity per day)* _____ NO

90% of injected dose excreted into urine within 48hours; 10% by feces within 48 hours. Excreted as intact hormone – not as water; not as CO_2

4. Are there documented human risks from exposure to the agent (risks may be determined from the MSDS or from references contained in the Biosafety Manual):

X YES *(Complete Questions 4a-4e)* _____ NO *(Skip to Question 5)*

4a. Indicate the route of human exposure:

_____ Inhalation
X Contact
X Ingestion
_____ Parenteral
_____ Other (describe below):

4b. Describe the risks associated with the agent (mutagen, teratogen, etc.):

Doses are extremely low thereby limiting risks significantly

4c. Indicate the acutely toxic/infectious dose in humans (in mg/kg or number of organisms):

The ALI for tritium is 80mCi; this allowable level of ingestion is greatly in excess of the dose being injected which is approximately 0.02mCi total. Therefore should be no toxic/infectious dose.

4d. Describe any genetic changes to the organism and their suspected effects:

None

4e. Describe the symptoms of exposure:

Nausea and vomiting are usually the initial symptoms of a mile exposure. Radiation poisoning if person should ingest more than 80mCi of ^{3}H

4f. Describe the first aid methods to be taken in the case of exposure:

Radiation safety will immediately be contacted and EVMS guidelines for exposure as outlined in Appendix A Emergency Procedures will be followed. See attached to protocol or EVMS website

4g. Indicate all personal protection required:

X Lab coat/dedicated clothing
_____ Apron
X Gloves
X Face shield
X Goggles
X Respirator
_____ Other (Describe below):

5. Are there risks to other animals in the room or in the animal facility?

X YES *(Complete Questions 5a-5e)* _____ NO *(Skip to Question 6)*

5a. Describe the risk to other animals:

Exposure to radioactive material (minimal to none)

5b. Indicate the route of animal exposure:

Ingestion

5c. Describe all methods that will be used to contain the risk factor:

Animals under isotope study will be confined to single housing with no tactile contact with other animals until isotope has cleared animals system (usually within 72 hours) and exposure is eliminated.

5d. Are special animal care requirements necessary?

_X___ YES *(Describe below)* _____ NO

Please see EVMS Radiation Safety manual section 14. Urine and feces will be collected until isotope has cleared the animals system. Animal/s under study cannot be pair housed or close to tactile contact with another animal. Animal does NOT have to be quarantined or isolated.

5e. Are special waste or carcass disposal requirements necessary?

_X___ YES *(Describe below)* _____ NO

Please see EVMS Radiation Safety manual section 13.2 and section 14

6. Briefly describe the procedure(s) for administration of the agent and how the agent is metabolized and/or excreted. Include how long the agent is expected to remain in/be shed from the animal, concentration of agent in any specific organs, etc.

Radioactive hormones are administered IV via a maternal saphenous vein for 60minutes at ≈ dosage of 10μCi ^{3}H steroid (total 20μCi) total. Blood samples which are collected at 0, 50, 55, and 60 minutes are used to determine clearance rates and interconversion of ^{3}H isotope labeled hormones (cortisol, cortisone, testosterone, androstenedione) Steroid hormones (^{3}H labeled as well) are excreted without further breakdown and because of locations of ^{3}H in the molecule, no water or $^{14}CO_2$ is formed. Steroids are cleared from the animal via urine (90%) and feces (10%) within 48-72hours.

6a. Have all laboratory personnel and CompMed staff been instructed of these hazards and received training on proper handling techniques?

_X___ Laboratory Personnel _X___ CompMed Staff

7. Please provide any other relevant information regarding the agent (e.g., unique containment recommendations): N/A

Eastern Virginia Medical School
Institutional Animal Care and Use Committee

Attachment E: Animal Surgery

Project Title: Regulation of Fetal-Placental Development in the Primate

A. PRE-OPERATIVE PROCEDURE

All animal activity proposals involving surgery must provide specific details of pre- through post-procedural care and relief of pain and distress.

1. List by name the person(s) who will be responsible for evaluating the health status of the animals?

   Attending and or Clinical Institutional Veterinarian
   Gerald J. Pepe, Ph.D.
   [redacted]

2. Will food be withheld?

   X YES *(Please explain below and indicate how long food will be withheld)* _____ NO

   Food will be removed late afternoon before surgical procedures scheduled for the following morning. Morning chow will be withheld until after surgery.

3. List all pre-operative anesthetics/analgesics to be used:

   Chemical restraint will be achieved with ketamine HCl (5-10 mg/kg). Glycopyrrolate (0.004-0.008 mg/kg) will also be administered prior to surgery.

4. Describe briefly how animals will be prepared for surgery:

   Surgery will be performed aseptically. Chemical restraint will be accomplished with ketamine. The animal's abdomen, lower legs, and a portion of the back skin (for grounding cautery unit) and forearm will be shaved. Venous access will be established via brachial vein for administration of IV fluids (Lactated Ringers or 0.09% Sodium Chloride). The animal will be intubated, and isoflurane anesthesia will be established. Monitoring of blood oxygen saturation, heart rate, and blood pressure will be initiated. The animal's abdomen will be prepared for surgery by passing iodine-soaked gauze sponges in a rotating pattern, alternating with isopropyl alcohol. This will be done a minimum of 3 times to assure that the skin is as disinfected as possible. The animal will be covered with a sterile surgical drape to establish a sterile field around the abdomen. A catheter is inserted into a saphenous vein and fluids (5% dextrose-water) administered. An initial blood sample obtained from the maternal saphenous vein is analyzed for blood chemistries (e.g. pH, pCO_2, pO_2, HCO_3, oxygen saturation; glucose, Na, K, Cl, etc.) using an I-Stat system. The surgery is a sterile procedure.

B. ANESTHETIC PROCEDURE

5. Will animals be anesthetized?

   X YES *(Complete Questions 6-8)* _____ NO *(Explain below, then skip to Section C)*

6. Who will administer the anesthesia?

   A trained member of the CompMed staff will be primarily responsible for anesthesia. When a CompMed staff member is not available, [redacted] or [redacted] will administer anesthesia.

7. What anesthetic will be used (name and dosage) and how will it be administered? Who will keep records?

Anesthesia will be achieved with isoflurane gas vaporized with a MAC of 1-2% in 100% oxygen (inhaled). Isoflurane is provided by CompMed, and CompMed will keep anesthesia records. Blood chemistry will be determined using I-Stat instrumentation (pH, pCO_2, pO_2, saturation, Hg, HCO_3, base deficit, etc) which is the property of Dr. Pepe. All records will be available to Comparative Medicine as needed.

8. Explain how anesthetic recovery will be monitored and indicate the person(s) responsible for monitoring the recovery.

The animal will remain on the heated OR table until returned to her cage. The animal will be extubated in the OR when swallowing reflex is observed. She will be returned to her cage when both blinking and swallowing reflexes are observed. Monitoring will be documented at least every 10-15 minutes until the animal is alert and able to maintain a sitting position (Stage 2). Monitoring will be conducted in accordance with IACUC policy and will change if IACUC policy changes. The anesthetist is responsible for monitoring immediate postoperative recovery and documenting recovery using a form developed in consultation with CompMed. The anesthetist will consult with a member of the CompMed staff or the Institutional Veterinarian if problems arise.

C. POST-OPERATIVE PROCEDURE:

Investigators should refer to the EVMS Post-Operative Care Guidelines when developing post-operative procedures.

"Appropriate post-operative records must be maintained in accordance with professionally accepted veterinary procedures regardless of the location of the animal. The attending veterinarian retains the authority to change post-operative care as necessary to ensure the comfort of the animal." AWA, Section 13 and 9 CFR, April 14, 1997.

9. Who will monitor post-operative care on a daily basis?

[redacted] (Animal Coordinator), the PI, or a trained member of the PI staff will monitor recovery according to IACUC policy and will consult with a member of the CompMed staff or the Institutional Veterinarian if the animal appears to be in pain or if complications occur.

10. Who will keep the post-operative record and where will the records be maintained?

Recovery will be documented on forms developed in consultation with the Institutional Veterinarian. These forms will be added to the animal's permanent record maintained by CompMed in the [redacted] floor animal facility.

11. Will post-operative analgesics be administered?

X YES _____ NO (*Explain below, then skip to Section D*):

12. Provide the following information about post-operative analgesia administration:

Agent: Buprenorphine

Dose and Route: 0.03 mg/Kg BW (IM) Frequency: At surgery and 6-8hours postoperatively, 3days BID followed PRN up to 5days

Post-Operative Duration of Care: 7days of monitoring

Agent: Ketoprofen

Dose and Route: 2 mg/BW (IM/oral) Frequency: SIB 3Days; at surgery and day 2 and 3 postoperatively

Post-Operative Duration of Care: 7days of monitoring

D. MULTIPLE SURVIVAL SURGERY

All multiple survival surgery must be conducted in accordance with EVMS' Multiple Survival Surgery Policy.

The Animal Welfare Act does not allow for animals to undergo more than one (1) survival surgery unless scientifically justified by the principal investigator, appropriate for the animal's veterinary care, or approved by the USDA administrator. The IACUC must specifically review and approve every additional surgery to be performed on a single animal.

13. Will the animal be subjected to more than one survival surgery?

X YES *(Answer Questions 13a-13b)* _____ NO *(Skip to Question 14)*

13a. Please briefly outline the procedures, explain how the surgeries are related and justify the need for more than one surgery per animal:

The protocol is designed to elucidate the role of estrogen on placental fetal development and function and impact on adrenocortical self-sufficiency in the perinatal period and reproductive function in adulthood. Thus surgeries are related to each other both by development and by estrogen. Thus, we study the animal discrete times in control (no treatments) pregnancy, e.g. days 60, 100, 110, 120, 140 and 160/165 a time during which there is increased endogenous production of estrogen by the placenta. We then examine in that same animal the effect of removing estrogen (e.g. between day 100 and 170) or giving estrogen earlier (day 25-59). We also need to have a control for our drug manipulations and thus in animals in which estrogen is depleted by treatment with Letrozole, the animal is also studied following treatment with Letrozole and estradiol. Thus, each animal essentially serves as its own control. The major survival surgery performed is a cesarean section. On day 54, 60, 100, 110, 140 or 165 of gestation, baboons are briefly restrained in home cage via squeeze mechanism, injected with ketamine (10 mg/kg), intubated, anesthetized with isoflurane/oxygen and vitals (e.g. heart rate, blood pressure) monitored by CompMed staff. A 22g catheter is placed in the brachial vein and IV fluids administered (Lactated Ringers Solution). A second catheter is placed in the saphenous vein using a 19g catheter 24inches in length and 5% dextrose fluids administered. The animal's abdomen/surgical site is shaved and scrubbed with alcohol and Betadine solution. The animal is draped using sterile technique. Blood samples are obtained from the mother at '0' time, mid procedure and post placental delivery via saphenous catheter. Prior to surgery, a proper plane of anesthesia will be assured by lack of response to a noxious stimulus such as toe pinch. A vertical mid-line incision is made using a 10blade. Once the abdomen is open, the uterus is externalized and examined for placenta location. Mother's ovaries are examined for evidence of ovulation/corpus luteum. Two to four uterine vein samples are taken (3-5 ml) using a 23g needle. Fetal crown is localized and a horizontal incision is made in the uterus to remove the fetus. Amniotic fluid is recovered using a syringe. Once the fetus is carefully delivered, umbilical vein and artery samples are taken using a 23g butterfly set (< 1 ml on day 54-60; 1-2 ml on day 100 and 4-6 ml on day 170) for hormone, steroid and blood gas analysis. The umbilical cord is double clamped to ensure the safety of the mother; the fetus is then euthanized by injecting via the umbilical artery Fatal Plus euthanasia solution. After the fetus expires (no heart beat), the cord is cut, placenta is delivered. Segments of the placenta and the fetal adrenal, hypothalamus, pituitary gland, lungs, kidneys, liver, skeletal muscle, pancreas, subcutaneous and visceral fat, and gonads are collected, portions fixed in formalin or snap frozen for subsequent immunocytochemical-biochemical/ mRNA determinations. The uterus is cleaned and closed using 2-0 PDS II suture. The uterus is manually messaged to stimulate contractions and shut down bleeding. Once closed, the uterus is rinsed with sterile saline and placed back in the abdomen. The abdomen is then rinsed with sterile saline to remove any blood clots. Prior to closing the abdomen, a small 10-15gram sample of visceral/abdominal fat is ligated and removed for RNA analysis. The abdomen is closed by three layers; the first layer (peritoneum) is closed using 2-0 PDS II simple interrupted stitch. If present, a second layer (fascia) is closed using 2-0 Dexon II suture. If a clear fascia layer is not present then a SQ layer is closed using a continuous stitch. Finally, bupivacaine is applied topically prior to closing the third and final layer. The skin is closed using 3-0 PDS II. Vet-bond adhesive glue is applied to the incision line once skin is closed. Since dissolvable suture material is used, no suture removal is required The mother is injected with 10mg/kg IronDextran IM for iron supplementation. Buprenorphine 0.03mg/kg and Ketoprofen 2mg/kg is given IM during immediate recovery. Isoflurane is shut off and animal is monitored until swallowing is present. Once able, the animal is extubated and continues to be monitored in home cage until fully upright and responsive to stimuli. Immediate post-op monitoring is done by Com Med staff using procedures outlined in the EVMS Post-Operative Care Guidelines Manual and in Attachment E below. PI staff is also present during immediate recovery. In selected experiments, the fetus is delivered live either spontaneously (on day 184) or by cesarean section on day 170 and reared to adulthood.

Animals reared at EVMS from neonate to adulthood, once puberty has been reached and normal menstrual cycles exhibited, will be hemi-ovariectomized. These animals will be studied for Gonadotropin Challenge Test / Implantation of indwelling cannulas.

13b. How many surgeries will each animal experience?

Each animal may undergo up to six (6) major survival surgeries without complications to the animal. While this is the optimal number to achieve statistically valid data, we work closely with the EVMS veterinarian to be sure that animals are healthy and have no untoward medical and/or behavioral complications (adhesions; uterine windows etc.) that would not be compatible with performing another surgery.

14. Have any animals to undergo major survival surgery in this protocol already undergone major survival surgery in any other IACUC protocols?

_____ YES *(Answer Questions 14a-14d)* _X_ NO *(Skip to Section E)*

14a Identify all animals that have undergone prior surgical procedures in another protocol:

14b. Identify all of the previous procedure(s) involved:

14c. Identify the IACUC protocol number(s) the previous surgeries were performed under:

14d. In accordance with the Animal Welfare Act, the IACUC must specifically review and approve all requests for the use of animals that have undergone previous survival surgery under another IACUC protocol. Please justify the need to reutilize such animals in this surgical protocol:

E. SURGICAL PROCEDURES:

15. Is the surgical procedure to be addressed on this page a major surgical procedure or a minor surgical procedure (a major surgical procedure is defined as involving penetration of a body cavity and/or resulting in a disability or dysfunction in the animal):

_____ Major surgical procedure _X_ Minor surgical procedure

16. Is this survival surgery?

_X_ YES _____ NO

17. Indicate what number the surgery described on this page is (i.e., only procedure to be performed, 1st surgical procedure, 2nd surgical procedure, etc.) If you are performing the same exact surgical procedure more than once on a single animal, indicate below how many times you will be performing this procedure on the animal (in this case, you will only need to submit one copy of this page for that specific procedure):

The procedure to be performed is an amniocentesis between day 80-95 of gestation. Amniotic fluid is obtained for fetal sex determination.

18. Indicate the location (provide a specific room number if surgery will be conducted outside of the

Surgical procedures will be performed in an approved nonhuman primate surgical suite located in the CompMed vivarium between 8 am and 4 pm, Monday-Friday.

19. Describe the entire surgical procedure:

Objective is to determine the sex of the fetus. Briefly, on day 90 of gestation, pregnant baboons are sedated with ketamine, and anesthetized with isoflurane using a nose cone. The fetus, fetal head, limbs etc and placenta are localized by ultrasound (not sterilized). A sterile 22 gauge needle (10 ml syringe attached) is then inserted through the abdomen and uterine wall and into the amniotic fluid away from the fetus/placenta and approximately 5-10 ml of amniotic fluid is removed. Fetal heart rate is monitored before and after the procedure to ensure that the fetus has not been compromised. The animal is returned to its cage and monitored by staff in Dr. Pepe's laboratory to Stage 0.

E. SURGICAL PROCEDURES:

15. Is the surgical procedure to be addressed on this page a major surgical procedure or a minor surgical procedure (a major surgical procedure is defined as involving penetration of a body cavity and/or resulting in a disability or dysfunction in the animal):

_X_ Major surgical procedure _____ Minor surgical procedure

16. Is this survival surgery?

_X_ YES _____ NO

17. Indicate what number the surgery described on this page is (i.e., only procedure to be performed, 1st surgical procedure, 2nd surgical procedure, etc.) If you are performing the same exact surgical procedure more than once on a single animal, indicate below how many times you will be performing this procedure on the animal (in this case, you will only need to submit one copy of this page for that specific procedure):

The only surgery to be performed in pregnant baboons is a cesarean section. No other surgeries are performed on pregnant animals. Cesarean sections can be performed up to six times on one animal as long as no adverse health changes occur in the animal(inability to sustain pregnancy, repeated failure to become pregnant, unhealthy uterus) This would be determined with PI/Vet consultations and could be cause for early termination.

18. Indicate the location (provide a specific room number if surgery will be conducted outside of the Comp Med animal facility, what time of day and what day(s) of the week surgery will be performed. Take into consideration the time necessary for care after anesthesia and surgery:

Surgical procedures will be performed in an approved nonhuman primate surgical suite located in the CompMed vivarium between 8 am and 4 pm, Monday-Friday

19. Describe the entire surgical procedure:

To determine the role of estrogen on placental-fetal development and impact on fertility in adulthood. On day 54, 60, 100, 110, 140 or 165 of gestation, baboons are briefly restrained in home cage via squeeze mechanism, injected with ketamine (10 mg/kg), intubated, anesthetized with isoflurane/oxygen and vitals (e.g. heart rate, blood pressure) monitored by CompMed staff. A 22g catheter is placed in the brachial vein and IV fluids administered (Lactated Ringers Solution). A second catheter is placed in the saphenous vein using a 19g catheter 24inches in length and 5% dextrose fluids administered. The animal's abdomen/ surgical site is shaved and scrubbed with alcohol and Betadine solution. The animal is draped using sterile technique. Blood samples are obtained from the mother at '0' time, mid procedure and post placental delivery via saphenous catheter. Prior to surgery, a proper plane of anesthesia will be assured by lack of response to a noxious stimulus such as toe pinch. A vertical mid-line incision is made using a 10blade. Once the abdomen is open, the uterus is externalized and examined for placenta location. Mother's ovaries are examined for evidence of ovulation/corpus luteum. Two to four uterine vein samples are taken (3-5 ml) using a 23g needle. Fetal crown is localized and a horizontal incision is made in the uterus to remove the fetus. Amniotic fluid is recovered using a syringe. Once the fetus is carefully delivered, umbilical vein and artery samples are taken using a 23g butterfly set (< 1 ml on day 54-60; 1-2 ml on day 100 and 4-6 ml on day 170) for hormone, steroid and blood gas analysis. The umbilical cord is double clamped to ensure the safety of the mother; the fetus is then euthanized by injecting the umbilical artery with Fatal Plus euthanasia solution. After the fetus expires (no heart beat), the cord is cut, placenta is delivered. Segments of the placenta and the fetal adrenal, hypothalamus, pituitary gland, lungs, kidneys, liver, skeletal muscle, pancreas, subcutaneous and visceral fat, and gonads are collected, portions fixed in formalin or snap frozen for subsequent immunocytochemical-biochemical/ mRNA determinations. The uterus is cleaned and closed using 2-0 PDS II suture. The uterus is manually messaged to stimulate contractions and shut down bleeding. Once closed, the uterus is rinsed with sterile saline and placed back in the abdomen. The abdomen is then rinsed with sterile saline to remove any blood clots. Prior to closing the abdomen, a small 10-15gram sample of visceral/abdominal fat is ligated and removed for RNA analysis. The abdomen is closed by three layers; the first layer (peritoneum) is closed using 2-0 PDS II simple interrupted stitch. If present, a second layer (fascia) is closed using 2-0 Dexon II suture. If a clear fascia layer is not present then a SQ layer is closed using a continuous stitch. Finally, bupivacaine is applied topically prior to closing the third and final layer. The skin is closed using 3-0 PDS II. Vet-bond adhesive glue is applied to the incision line once skin is closed. Since dissolvable suture material is used, no suture removal is required. The mother is injected with 10mg/kg IronDextran IM for iron supplementation. Buprenorphine 0.03mg/kg and Ketoprofen 2mg/kg is given IM during immediate recovery. Isoflurane is shut off and animal is monitored until swallowing is present. Once able, the animal is extubated and continues to be monitored in home cage until fully upright and responsive to stimuli. Immediate

post-op monitoring is done by Com Med staff using procedures outlined in the EVMS Post-Operative Care Guidelines Manual and in Attachment E below. PI staff is also present during immediate recovery. In selected experiments, the fetus is delivered live either spontaneously (on day 184) or by cesarean section on day 170 and reared to adulthood.

In some experiments (day 165-175 only), the fetus-neonate is not injected with pentobarbital since it will be reared to adulthood. Live fetuses are cleared of mucous, stimulated to breathe, placed in a warm blanket until returned to the mother for sub-sequent rearing to adulthood. If neonate is not received by the mother for unknown reasons and fails to thrive, the neonate will be hand reared until old enough/strong enough to be introduced to age appropriate conspecies. In some cases, a surrogate mother can be used to nurse the neonate. This is the best cases.

E. SURGICAL PROCEDURES:

15. Is the surgical procedure to be addressed on this page a major surgical procedure or a minor surgical procedure (a major surgical procedure is defined as involving penetration of a body cavity and/or resulting in a disability or dysfunction in the animal):

_X___ Major surgical procedure _____ Minor surgical procedure

16. Is this survival surgery?

_X___ YES _____ NO

17. Indicate what number the surgery described on this page is (i.e., only procedure to be performed, 1st surgical procedure, 2nd surgical procedure, etc.) If you are performing the same exact surgical procedure more than once on a single animal, indicate below how many times you will be performing this procedure on the animal (in this case, you will only need to submit one copy of this page for that specific procedure):

The 1st survival surgery to be performed in adolescent baboons is removal of one ovary (hemi-ovariectomy) at approximately 96 months of age (i.e. after fertility testing by breeding). These animals, born to mothers untreated or treated *in utero* with Letrozole or Letrozole plus estradiol, will have had no prior major surgeries. *In vivo* response of the remaining ovary to pitultary gonadotropin will be initiated approximately 9 months after hemi-ovariectomy and animals have exhibited at least three consecutive normal menstrual cycles. Gonadotropin challenge studies will require implantation of indwelling cannulas for constant infusion of pituitary hormones over a 20-day period.

18. Indicate the location (provide a specific room number if surgery will be conducted outside of the Comp Med animal facility, what time of day and what day(s) of the week surgery will be performed. Take into consideration the time necessary for care after anesthesia and surgery:

Surgical procedures will be performed in an approved nonhuman primate surgical suite located in the CompMed vivarium between 8 am and 4 pm, Monday-Friday

19. Describe the entire surgical procedure:

Objective is to determine the role of estrogen *in utero* on ovarian development and fertility in adulthood. Juvenile to adult baboons from experimental group #12 are briefly restrained in home cage via squeeze mechanism, sedated with ketamine (10mg/kg), intubated, anesthetized with isoflurane/oxygen and vitals (e.g. blood pressure) monitored by CompMed staff. A 22g catheter is placed in the brachial vein and IV fluids administered (Lactated Ringers Solution). A second catheter is placed in the saphenous vein using a 19g catheter 24inches in length and 5% dextrose fluids administered. The animals' abdomen/surgical site is shaved and scrubbed with alcohol and Betadine solution. The animal is draped using sterile technique. Prior to surgery, a proper plane of anesthesia will be assured by lack of response to a noxious stimulus such as toe pinch After making a small abdominal incision a retractor is put in place, lap sponge can be used if needed for a clearer view, ovaries are located. Once the ovulation site is located, the ovarian ligament and vasculature is identified, clamped and cauterized and the ovary removed. Once removed, clamps are carefully removed and ligament/cautery site observed for bleeding. Abdomen is rinsed with sterile saline. Abdominal layers are closed (peritoneum, fascia/SQ and skin) using 2-0 PDS and 2-0 Dexon. Bupivacaine applied topically prior to skin closure. Vet-bond skin adhesive applied to the incision site once closed. Since dissolvable suture material is used, no suture removal is required Buprenorphine 0.03mg/kg and Ketoprofen 2mg/kg is given IM during immediate recovery. Isoflurane is shut off and animal is monitored until swallowing is present. Once able, the animal is extubated and continues to be monitored in home cage until fully upright and

responsive to stimuli. Immediate post-op monitoring is done by Com Med staff using procedures outlined in the EVMS Post-Operative Care Guidelines Manual and in Attachment E below. PI staff is also present during immediate recovery. Animals will be treated postoperatively with analgesics and recovery monitored as outlined in the Attachment E below. *In vivo* responsivity of the remaining ovary to pituitary gonadotropin will be initiated approximately 6-9 months later and after animals have exhibited at least three consecutive normal menstrual cycles. Gonadotropin challenge studies will require implantation of indwelling cannulas for constant infusion of pituitary hormones over an extended period.

E. SURGICAL PROCEDURES:

15. Is the surgical procedure to be addressed on this page a major surgical procedure or a minor surgical procedure (a major surgical procedure is defined as involving penetration of a body cavity and/or resulting in a disability or dysfunction in the animal):

_X___ Major surgical procedure _____ Minor surgical procedure

16. Is this survival surgery?

_X___ YES _____ NO

17. Indicate what number the surgery described on this page is (i.e., only procedure to be performed, 1st surgical procedure, 2nd surgical procedure, etc.) If you are performing the same exact surgical procedure more than once on a single animal, indicate below how many times you will be performing this procedure on the animal (in this case, you will only need to submit one copy of this page for that specific procedure):

2nd survival surgery to be performed in adolescent baboons is the Gonadotropin challenge studies which requires the implantation of indwelling cannulas for constant infusion of pituitary hormones over a 20-day period.

18. Indicate the location (provide a specific room number if surgery will be conducted outside of the Comp Med animal facility, what time of day and what day(s) of the week surgery will be performed. Take into consideration the time necessary for care after anesthesia and surgery:

Surgical procedures will be performed in an approved nonhuman primate surgical suite located in the CompMed vivarium between 8 am and 4 pm, Monday-Friday

19. Describe the entire surgical procedure:

On experimental day 0, baboons will be sedated with ketamine, anesthetized with isoflurane/oxygen, intubated and an IV catheter placed in the brachial vein for IV fluid administration. Vitals are monitored (PO2, blood pressure, HR, resp, and blood gas). Neck and back (between the shoulder blades) are shaved and scrubbed for sterile procedure. Prior to the incision, a proper plane of anesthesia will be assured by lack of response to a noxious stimulus such as toe pinch. A small incision is made in the neck to expose the right jugular. A cannula of relatively small length is inserted into the right jugular vein and secured using suture. Implantation into the jugular vein permits use of a cannula smaller in length than if inserted into the femoral vessels. The cannula/PE tubing (0.030") will then be tunneled subcutaneously by way of sterile trochar to the scapular region and connected to ALZET osmotic minipump (Model 2ML1; 5.1 cm by 1.4 cm length/width; 5.1 grams weight; 6.5 ml volume) designed to deliver human LH or FSH (4.8 µg hLH/kg BW/day and 480 ng hFSH/kg BW/day) which is dissolved in sterile saline containing penicillin/streptomycin. A small incision is made in the scapular area to connect the pump to the PE tubing. Pump is implanted just under the skin. The exact dosage of hLH and/or hFSH infused may need to be altered slightly depending upon the LH and FSH level achieved in blood samples obtained 24 hrs after onset of infusion and is the reason human FSH (and LH) are used in this study since we can measure hLH and hFSH levels rapidly using our Diagnostic Immulite Automated RIA system. Both incisions are closed using 2-0 Dexon suture. Since dissolvable suture material is used, no suture removal is required. Bupivacaine applied topically prior to skin closure. Vet-bond skin adhesive applied to the incision site once closed. Buprenorphine 0.03mg/kg and Ketoprofen 2mg/kg is given IM during immediate recovery. Isoflurane is shut off and animal is monitored until swallowing is present. Once able, the animal is extubated and continues to be monitored in home cage until fully upright and responsive to stimuli. Immediate post-op monitoring is done by Com Med staff using procedures outlined in the EVMS Post-Operative Care Guidelines Manual and in Attachment E below. PI staff is also present during immediate recovery. Animals will be treated postoperatively with analgesics and recovery monitored as outlined in the Attachment E below.

E. SURGICAL PROCEDURES:

15. Is the surgical procedure to be addressed on this page a major surgical procedure or a minor surgical procedure (a major surgical procedure is defined as involving penetration of a body cavity and/or resulting in a disability or dysfunction in the animal):

_____ Major surgical procedure X Minor surgical procedure

16. Is this survival surgery?

X YES _____ NO

17. Indicate what number the surgery described on this page is (i.e., only procedure to be performed, 1st surgical procedure, 2nd surgical procedure, etc.) If you are performing the same exact surgical procedure more than once on a single animal, indicate below how many times you will be performing this procedure on the animal (in this case, you will only need to submit one copy of this page for that specific procedure):

3rd minor procedure to be performed in adolescent baboons. ALZET pump is changed out with a higher dose of FSH and LH being infused.

18. Indicate the location (provide a specific room number if surgery will be conducted outside of the Comp Med animal facility, what time of day and what day(s) of the week surgery will be performed. Take into consideration the time necessary for care after anesthesia and surgery:

Surgical procedures will be performed in an approved nonhuman primate surgical suite located in the CompMed vivarium between 8 am and 4 pm, Monday-Friday

19. Describe the entire surgical procedure:

After 7-10 days of infusion, (i.e. experimental day 17-20), the pump will be replaced with another Model 2ML1 pump containing the same amount of LH and a dose of FSH 1.25 times greater than that ultimately delivered during the previous 7 day period (i.e. approximately 600 ng FSH/kg BW/day). Baboons will be sedated with ketamine, anesthetized with isoflurane/oxygen, intubated and an IV catheter placed in the brachial vein for IV fluid administration. Vitals are monitored (PO_2, blood pressure, HR, resp, and blood gas). Scapular area scrubbed and prepped for ALZET pump replacement. Once the new pump is in place under the skin, 3-0 Dexon suture is put in place. Since dissolvable suture material is used, no suture removal is required. Bupivacaine applied topically prior to skin closure. Vet-bond skin adhesive applied to the incision site once closed. Bupivacaine applied topically prior to skin closure. Vet-bond skin adhesive applied to the incision site once closed. Ketoprofen 2mg/kg is given IM during immediate recovery. Isoflurane is shut off and animal is monitored until swallowing is present. Once able, the animal is extubated and continues to be monitored in home cage until fully upright and responsive to stimuli. Immediate post-op monitoring is done by CompMed staff using procedures outlined in the EVMS Post-Operative Care Guidelines Manual and in Attachment E below. PI staff is also present during immediate recovery. Animals will be treated postoperatively with analgesics and recovery monitored as outlined in the Attachment E below. Blood samples (3-5 ml) will continue to be collected during the study period under ketamine sedation.

E. SURGICAL PROCEDURES:

15. Is the surgical procedure to be addressed on this page a major surgical procedure or a minor surgical procedure (a major surgical procedure is defined as involving penetration of a body cavity and/or resulting in a disability or dysfunction in the animal):

X Major surgical procedure _____ Minor surgical procedure

16. Is this survival surgery?

_____ YES X NO

17. Indicate what number the surgery described on this page is (i.e., only procedure to be performed, 1st surgical procedure, 2nd surgical procedure, etc.) If you are performing the same exact surgical procedure more than once on a single animal, indicate below how many times

you will be performing this procedure on the animal (in this case, you will only need to submit one copy of this page for that specific procedure):

4th surgical procedure to be performed in adolescent baboons.

18. Indicate the location (provide a specific room number if surgery will be conducted outside of the Comp Med animal facility, what time of day and what day(s) of the week surgery will be performed. Take into consideration the time necessary for care after anesthesia and surgery:

Surgical procedures will be performed in an approved nonhuman primate surgical suite located in the CompMed vivarium between 8 am and 4 pm, Monday-Friday

19. Describe the entire surgical procedure:

This is the 2nd half of the study. This is a terminal procedure. The animal is prepped as outlined: Baboons will be sedated with ketamine, anesthetized with isoflurane/oxygen, intubated and an IV catheter placed in the brachial vein for IV fluid administration. Vitals are monitored (PO2, blood pressure, HR, resp, and blood gas). The animal is draped using sterile technique. Prior to the incision, a proper plane of anesthesia will be assured by lack of response to a noxious stimulus such as toe pinch. After making a small abdominal incision a retractor is put in place, lap sponge can be used if needed for a clearer view, ovaries are located. Once the ovulation site is located, the ovarian ligament and vasculature is identified, clamped and cauterized and the ovary removed. At this time the animal will be euthanized using Fatal Plus euthanasia solution and following EVMS guidelines. Remaining tissues will be collected and the animal disposed of under CompMed guidelines.

OFFICE OF RESEARCH | WWW.EVMS.EDU
[redacted] 22507 | Phone: 75[redacted] | Fax: 75[redacted]

EVMS
Eastern Virginia Medical School

October 11, 2011

Gerald J. Pepe, Ph.D.
Chair, Department of Physiological Sciences
Eastern Virginia Medical School
[redacted]
Norfolk, Virginia 23507

Dear Dr. Pepe: Jerry

The Institutional Animal Care and Use Committee reviewed your amendment request (*i.e., addition of six female baboons to be subjected to the procedures outlined in the approved protocol*) to the protocol entitled, ***Regulation of Fetal-Placental Development in the Primate*** **(IACUC #09-007)**, at its October 6, 2011 meeting. **The amendment to the protocol was approved.**

PLEASE NOTE: Although the Division of Comparative Medicine (CompMed) will make every effort to provide housing, husbandry, and veterinary support for your research project, IACUC approval of this amendment request does not guarantee that CompMed will be able to accommodate the work immediately. Available space and resources within the CompMed animal facility are determined by the number of ongoing projects and the animal census at any given time. Please consult with the CompMed management team to confirm availability prior to implementing the approved modification(s).

It is the responsibility of the Principal Investigator to notify the Committee, in writing, of any deviation from the IACUC-approved protocol. Please note that a change in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian prior to submitting the amendment request to the IACUC for approval.

It is also the responsibility of the Principal Investigator to provide for regular observation of the health of the research animals and to provide the Division of Comparative Medicine (CompMed) with the name(s) and telephone number(s) of the person(s) who may be contacted and/or take action in the event that the CompMed staff notes an emergency condition with the research animals.

Thank you for your continued cooperation with the Institutional Animal Care and Use Committee.

Sincerely,

[redacted]
[redacted] *Chair*
Institutional Animal Care and Use Committee

[redacted]

cc: [redacted]
Attending Veterinarian
Division of Comparative Medicine

[redacted]
Program Manager
Division of Comparative Medicine

[redacted]
Associate Dean for Research
Institutional Official

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[redacted] Norfolk, VA 23507 | Phone: [redacted] | Fax: [redacted]

EVMS
Eastern Virginia Medical School

August 17, 2011

Gerald J. Pepe, Ph.D.
Chair, Department of Physiological Sciences
Eastern Virginia Medical School
[redacted]
Norfolk, Virginia 23507

Dear Dr. Pepe: Jerry

The Institutional Animal Care and Use Committee (IACUC) has reviewed the requested information regarding your request to amend the protocol entitled, ***Regulation of Fetal-Placental Development in the Primate*** **(IACUC #09-007).** Your original request was reviewed by the IACUC at its June 2, 2011 meeting. **The following amendments to the protocol have been approved via the facilitated review process:**

1. **addition of [redacted] to perform Doppler analyses and to train your laboratory staff to perform the procedure,**
2. **addition of non-pharmaceutical-grade Serotonin to test the impact of uterine spiral artery invasion on utero-placental blood flow dynamics and blood flow/cardiovascular function in the non-human primate neonate and adolescent,**
3. **addition of Ketamine sedation and Isoflurane anesthesia for the Serotonin challenge procedure, and**
4. **addition of Terbutaline as needed to treat fetal brachycardia.**

It is the responsibility of the Principal Investigator to notify the Committee, in writing, of any deviation from the IACUC-approved protocol. Please note that a change in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian prior to submitting the amendment request to the IACUC for approval.

It is also the responsibility of the Principal Investigator to provide for regular observation of the health of research animals and to provide the Division of Comparative Medicine (CompMed) with the name(s) and telephone number(s) of the person(s) who may be contacted and/or take action in the event that the CompMed staff notes an emergency condition with the research animals.

Thank you for your continued cooperation with the Institutional Animal Care and Use Committee.

Sincerely,

[redacted]

[redacted] *Chair*
Institutional Animal Care and Use Committee

[redacted]

cc: [redacted]
Attending Veterinarian
Division of Comparative Medicine

[redacted]
Project Manager
Division of Comparative Medicine

[redacted]
Associate Dean for Research
Institutional Official

April 2010
Updated February 2011

RECEIVED
APR 25 2011
Office: 757-[redacted]
[redacted] EVMS

REQUEST TO AMEND A PREVIOUSLY APPROVED IACUC PROTOCOL

Institutional Animal Care and Use Committee (IACUC)

Eastern Virginia Medical School
Office of Research

IACUC Office
[redacted]

FOR OFFICE USE ONLY
Date Received: 4/25/11 **Review Method:** X FCR / ___ FR / ___ Administrative (*Personnel Changes Only*)
IBC Approval? X Yes / ___ No **IBC Approval Date:** 5/26/11 **Final Approval Date:** 8/17/11 [redacted]

General Information and instructions:
1. All requested amendments must be approved by the IACUC **before** they are implemented.
2. Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian **before** the changes are submitted to the IACUC Office.
3. A request to increase the number of animals assigned to a protocol must be approved by the IACUC **before** the animals are ordered.
4. Submit the original signed amendment form, along with seventeen (17) photocopies, to the IACUC Office.
5. ***The IACUC reserves the right to require submission of an IACUC "Initial Review Form" based upon the Committee's assessment of the amendment(s) requested herein.***

I. ADMINISTRATIVE INFORMATION

Protocol Number: 09-007	**Protocol Initial Approval Date:** 06/~~2009~~ 7/13/09 [redacted]
Protocol Title: Regulation of Fetal-Placental Development in the Primate	
Principal Investigator: Gerald J Pepe	
Approved USDA Pain Code Level(s) – *not required for personnel additions only*: **B, C, and D**	

II. PERSONNEL

List all personnel to be added to or removed from the protocol. ***All persons authorized to work on the protocol must have completed the required CITI and OHSP training modules and must have completed a health/risk assessment with the Occupational Health Department.***

Name	Added (+) or Removed (-)	E-mail Address *(additions only)*	CITI Training Certification Number *(additions only)*	CITI Training Species Module(s) Completed *(additions only)*	OHSP Training Certification Number *(additions only)*	Occupational Health/Risk Assessment Date *(additions only)*
[redacted]	+	[redacted]@ EVMS.EDU	[redacted]	NHP	[redacted]	8/4/11 [redacted]

Procedure(s) to be performed by personnel addition #1:

Doppler analysis

Procedure(s) to be performed by personnel addition #2:

Procedure(s) to be performed by personnel addition #3:

III. PROPOSED CHANGES TO THE ORIGINALLY APPROVED PROTOCOL

☐ **A. CHANGE OF SPONSOR/FUNDING SOURCE**

List the new/approved funding source, the project/grant number, the project/grant title (if different from the IACUC protocol title), and the award period.

☐ **B. CHANGE IN PROJECT SITE(S)**

List all new locations (i.e., building and room number(s)) and indicate what procedure(s) will be performed in each new location.

☐ **C. CHANGE OF SPECIES AND/OR STRAIN**

List the species to be added to or removed from the protocol. Please provide the rationale for any species/strain additions and indicate the USDA Pain Category assignment for each new species/strain. Also, list any anticipated changes in veterinary care and/or husbandry due to the new species/strain.

Species and/or strain	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E

☐ **D. CHANGE IN ANIMAL NUMBERS**

List the number of animals to be added to or removed from the protocol. If animals will be added, please provide the justification for the increase and indicate the number of animals to be assigned to each USDA Pain Category.

Species and/or strain	Year (1, 2 or 3)	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E	TOTAL

Will the animals be ordered through CompMed? _____Yes _____ No ***(Identify the source and provide the rationale/justification below.)***

Will the animals require special housing (e.g., specific bedding requirements, isolator cages, special feed, etc.)
_____ Yes ***(Please specify below.)*** _____ No

USDA Pain Code B = *Breeding or holding colony; no animal manipulation.*
USDA Pain Code C = *Procedures involving no or momentary slight pain or distress for which no pain-relieving drugs are used.*
USDA Pain Code D = *Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.*
USDA Pain Code E = *Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.*

X **E. CHANGE IN EXPERIMENTAL PROCEDURES AND/OR INSTRUMENTATION**

Describe, in detail, the proposed change(s). *Please indicate potentially painful/distressing effects on the animals as a result of the proposed change(s). Also, please indicate any expected adverse effects and any changes in monitoring, husbandry, housing, early endpoint, and/or experimental endpoint. Finally, please complete the checklist below.*

In study three (3) of our current protocol #09-007, pregnant baboons are treated with nothing or estradiol (350 µg/kg maternal body wt) daily on days 25-59 of gestation, animals not further treated and then delivered at day 165 of gestation (term = day 184) or allowed to spontaneously deliver at term and raise the neonate. Using this treatment paradigm we have shown that a premature elevation in estradiol early in pregnancy regulates uterine spiral artery invasion and have proposed that the latter impacts utero-placental blood flow dynamics and blood flow/cardiovascular function in the neonate/adolescent. To test this hypothesis, we propose to determine basal (resting) and serotonin-induced uterine arterial and umbilical (fetal) arterial and fetal middle cerebral arterial blood flow dynamics as well as fetal heart rate using Doppler analysis at days 60, 100 and 165 of gestation and basal and serotonin-induced cardiovascular function in the neonate at 3, 6, 12, 24, 36 and 48 months of age. Studies have been initiated with Dr. Pepe's colleagues at the University of Maryland and who have demonstrated that umbilical artery blood flow at day 165 in animals treated with estradiol early in pregnancy was reduced 50% following maternal infusion of serotonin (5HT) at 4 µg/min/kg BW for 20 min and then at 8 µg/kg BW/min for an additional 20 mins and unaltered in animals untreated. We propose to perform similar studies here at EVMS and to also determine whether the impact on umbilical (fetal) blood flow is programmed in utero and thus remains problematic in the perinatal period and into adulthood. For intrauterine studies, baboons are sedated with ketamine (10mg/kg im) and prepped/monitored for the test under Comp Med SOP. An intracatheter is placed in the maternal saphenous vein with 0.9% NaCl drip at 1ml/min; a baseline blood sample (3 ml) obtained to determine blood chemistries (Na, K, etc), gases (pCO2, pO2) and acid/base status and subsequent analysis of estradiol, progesterone and androgens. Maternal heart rate, blood pressure and body temp are monitored by Comp Med throughout the procedure. Baseline measurements of fetal heart rate and utero-placental-fetal blood flow are measured using ultrasound and Doppler as established at the University of Maryland. Briefly, animals are infused with saline (0.5 ml/min) for 20 mins and fetal heart rate measured/monitored continuously and uterine, umbilical, and fetal middle cerebral arterial blood flow dynamics determined during the final 5 mins of infusion using Ultrasound/Doppler procedures. [redacted], Chair of the Department of OB/GYN EVMS, an acknowledged expert in utilization of Doppler for blood flow analyses in high risk pregnant women has agreed to perform these studies and train appropriate staff in Dr. Pepe's lab (e.g. [redacted] Assistant Professor of OB/GYN and Physiological Sciences, EVMS) for future studies. A maternal blood sample (2 ml) is also obtained during the blood flow analyses for determination of blood chemistries/gases. After collection of basal data, a maternal infusion of serotonin (4 µg/kg/min) is initiated and fetal heart continuously measured and blood flow/chemistry studies performed during the final 5 mins of this 20 min infusion. Upon completion, the dose of serotonin is increased to 8 µg/kg BW/min and blood flow/chemistry analyses determined as described. Infusion of serotonin will be stopped immediately should fetal heart rate decrease to 80 bpm. If fetal HR drops below 80 bpm for more than 3 minutes, terbutaline will be administered IV/SQ to the mother under direction of the attending veterinarian to alleviate the fetal bradycardia. If fetal HR does not return to normal and continues to drop or fetal demise occurs, a cesarean section will be performed as outlined in IACUC 09-007. Once the final dose of 5-HT is infused, the animal is monitored until recovered under Comp Med post op monitoring SOP and returned to home cage. The mother is being monitored for HR, BP and body temp throughout the procedure.

Following delivery of the neonate, starting at three (3) months of age, we propose to measure cardiovascular dynamics (blood pressure, heart rate and brachial artery diameter and flow) before and after occlusion and administration of serotonin at 4 and then 8 µg/kg BW/ min essentially as described for the in utero studies. Briefly; at 3 and 6 months postpartum, the mother is lightly sedated with ketamine im, the neonate removed, lightly sedated with ketamine, intubated and prepped by Comp Med following Comp Med SOP. At ages 12-48 months, adolescents are individually caged and comparably prepared for study as outlined. The neonate/adolescent is monitored for BP, HR and body temperature throughout the procedure and an IV catheter is placed in the saphenous vein, 0.9% NaCl drip in place. A baseline blood sample (2ml) is obtained for blood chemistry/gas/ acid: base analyses. Brachial artery flow and diameter are then determined at rest and 15 sec and 1 minute respectively after reactive hyperemia is induced by 5 minute cuff occlusion of the forearm as performed in human studies (Hamburg et al, Relation of brachial and digital measures of vascular function in the community, Hypertension, 57:390-396, 2011). Studies are then repeated after during the last 5 infusion of serotonin at 4 and then 8 µg/kg BW. Upon completion of the test, the neonate/adolescent is monitored post operatively following Comp Med SOP and returned to home cage. Any adverse effects will be addressed as determined by the attending veterinarian.

Please check all that apply to the proposed change(s). Complete the required IACUC Attachment and submit it (i.e., the original and 17 photocopies) along with the amendment form.	
Biohazardous Agents: ____ Yes X No (e.g., recombinant DNA, RNA, all tissue culture, laboratory-induced infection, cultured pathogens)	Complete and submit **Attachment D**
Radioisotopes, *in vivo*: ____ Yes X No	Complete and submit **Attachment D**
Chemical Agents: ____ Yes X No (e.g., known or suspected chemical hazards, mutagens, or teratogens)	Complete and submit **Attachment D**
Stress or Prolonged Restraint: ____ Yes X No	Complete and submit **Attachment C**
Food and/or Water Deprivation: ____ Yes X No	Complete and submit **Attachment C**
Surgical Procedures: ____ Yes X No (i.e., single survival, multiple survival, or non-survival)	Complete and submit **Attachment E**
Antibody Production: ____ Yes X No	Complete and submit **Attachment F**
Toxicity Testing (LD50): ____ Yes X No	Complete and submit **Attachment G**
Lasers or Penetrating Electromagnetic Radiation: __ Yes X No	
Collection of Tissues, Cells, or Organs: ____ Yes X No (Please explain below.)	
Tumor Transplantation or Induction: ____ Yes X No	
Special Diet: ____ Yes X No (Please explain below.)	
Other : (Please specify below.)	

X **F. CHANGE IN ANESTHESIA, THERAPEUTICS, AND/OR EXPERIMENTAL AGENTS**

	Dose (mg/kg)	Route of Administration	Frequency of Administration	
Sedatives/Tranquilizers				
Ketamine	10-15mg/kg	IM	chemical restraint for serotonin challenge	
Anesthetics - General				
Isoflurane	~2 %MAC	Inhalant	At time of serotonin challenge	
Anesthetics - Local				
Analgesics				
Antibiotics				
Miscellaneous				
Terbutaline		IV / SQ	PRN under Veterinarian consult	**PRN**

EXPERIMENTAL AGENTS:

Agent: Serotonin (5-HT) **Agent Vehicle:** 0.9% NaCl
Route/Site: IV Maternal **Volume per administration:** 2-16ug/kg in 'step-up' fashion
Frequency of administration: +/-D60, D100, D160-175 of gestation
Expected side effects and/or changes in animal behavior: No expected changes in behavior, no expected post procedural effects. Animals will be monitored and treated accordingly. Expected decrease in fetal HR, possible fetal demise. Cesarean section performed in case of fetal demise as IACUC 09-007 outlines. Bradycardia in fetus below 80bpm, infusion/test is stopped.

Agent: Serotonin (5-HT) **Agent Vehicle:** 0.9% NaCl
Route/Site: IV **Volume per administration:** 2-16ug/kg in 'step-up' fashion
Frequency of administration: Every 3-12months starting at 3months of age over 5 years not to total more than six (6) test.
Expected side effects and/or changes in animal behavior: No expected adverse effects. Any observed change in behavior will be addressed with Veterinarian consult.

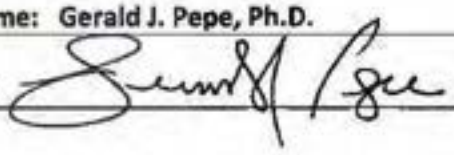
Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _____

INVESTIGATOR ASSURANCES:

☒	*I understand that it is my responsibility as the Principal Investigator to ensure that all personnel who work on this protocol are adequately trained. That training may be provided by me, an experienced member of the investigative staff, the Attending Veterinarian, a qualified Division of Comparative Medicine (CompMed) staff member, or an appropriate outside source. I hereby affirm that each person added to this protocol by way of this amendment request has been adequately trained to perform the procedure(s) indicated above. Any person who has not been adequately trained will be so trained prior to performing the procedure(s).*
☒	*No animal involved in this project will be subjected to discomfort, pain, or distress, unless it is unavoidable in the conduct of scientifically valuable research and approval of such has been approved by the IACUC.*
☒	*I agree to comply with all federal and institutional policies governing the use of animals used in this project.*

PRINCIPAL INVESTIGATOR:

Printed Name: Gerald J. Pepe, Ph.D.

Signature:  **Date:** 4/22/11

APPROVAL SIGNATURES:	
PRE-VETERINARY REVIEW: *Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian before the changes are submitted to the IACUC for review.* **Attending Veterinarian Printed Name:** [redacted] **Attending Veterinarian Signature:** [redacted]	**Date:** 7/19/11
FINAL IACUC APPROVAL: *All revisions must be approved by the IACUC prior to implementation.* **IACUC Chair or Vice Chair Printed Name:** [redacted] **IACUC Chair or Vice Chair Signature:** [redacted]	**Date:** 8/30/11

OFFICE OF RESEARCH WWW.EVMS.EDU
[redacted] Norfolk, VA 23507 Phone: [redacted] Fax: [redacted]

EVMS
Eastern Virginia Medical School

May 14, 2012

Gerald J. Pepe, Ph.D.
Chair, Department of Physiological Sciences
Eastern Virginia Medical School
[redacted]
Norfolk, Virginia 23507

Dear Dr. ~~Pepe~~ Jerry

The Institutional Animal Care and Use Committee has reviewed your request to amend the protocol entitled, ***Regulation of Fetal-placental Development in the Primate*** **(IACUC #09-007). The following amendments to the protocol have been approved via the facilitated review process:**

1. **permission to use gas anesthesia (i.e., Isoflurane) as the primary means of induction. It is the understanding of the Committee that Xylazine will be used as a secondary means of induction, as needed.**
2. **permission to add one extra day of videotape monitoring, to include 24 hours post-infusion.**

Although the Division of Comparative Medicine (CompMed) will make every effort to provide housing, husbandry, and veterinary support for your research project, IACUC approval of this amendment request does not guarantee that CompMed will be able to accommodate the work immediately. Available space and resources within the CompMed animal facility are determined by the number of ongoing projects and the animal census at any given time. Please consult with the CompMed management team to confirm availability prior to implementing the approved modification(s).

It is the responsibility of the principal investigator to notify the Committee, in writing, of any deviation from the IACUC-approved protocol. Please note that a change in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian prior to submitting the amendment request to the IACUC for approval.

It is also the responsibility of the principal investigator to provide for regular observation of the health of the research animals and to provide the Division of Comparative Medicine (CompMed) with the name(s) and telephone number(s) of the person(s) who may be contacted and/or take action in the event that the CompMed staff notes an emergency condition with the research animals.

Thank you for your continued cooperation with the Institutional Animal Care and Use Committee.

Sincerely,

[redacted]

[redacted] *Chair*
Institutional Animal Care and Use Committee

[redacted]

cc: [redacted]
Attending Veterinarian
Division of Comparative Medicine

[redacted]
Program Manager
Division of Comparative Medicine

[redacted]
Associate Dean for Research
Institutional Official

April 2010
Updated February 2011

REQUEST TO AMEND A PREVIOUSLY APPROVED IACUC PROTOCOL

Institutional Animal Care and Use Committee (IACUC)

Eastern Virginia Medical School
Office of Research

IACUC Office
[redacted]

Office: 757-[redacted]
Fax: 757-[redacted]

FOR OFFICE USE ONLY [redacted]

Date Received: 5/2/12 **Review Method:** ___ FCR / X FR / ___ Administrative (*Personnel Changes Only*)

IBC Approval? X Yes / ___ No **IBC Approval Date:** 7/12/12 **Final Approval Date:** 5/14/12 [redacted]

General Information and Instructions:

1. All requested amendments must be approved by the IACUC **before** they are implemented.
2. Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian **before** the changes are submitted to the IACUC Office.
3. A request to increase the number of animals assigned to a protocol must be approved by the IACUC **before** the animals are ordered.
4. Submit the original signed amendment form, along with seventeen (17) photocopies, to the IACUC Office.
5. ***The IACUC reserves the right to require submission of an IACUC "Initial Review Form" based upon the Committee's assessment of the amendment(s) requested herein.***

I. ADMINISTRATIVE INFORMATION

Protocol Number: 09-007	**Protocol Initial Approval Date:** 7/13/09
Protocol Title: Regulation of Fetal-Placental Development in the Primate	
Principal Investigator: Gerald J. Pepe	
Approved USDA Pain Code Level(s) – *not required for personnel additions only*: **B,C, and D**	

II. PERSONNEL

List all personnel to be added to or removed from the protocol. ***All persons authorized to work on the protocol must have completed the required CITI and OHSP training modules and must have completed a health/risk assessment with the Occupational Health Department.***

Name	Added (+) or Removed (-)	E-mail Address *(additions only)*	CITI Training Certification Number *(additions only)*	CITI Training Species Module(s) Completed *(additions only)*	OHSP Training Certification Number *(additions only)*	Occupational Health/ Risk Assessment Date *(additions only)*
1.						
2.						
3.						

Procedure(s) to be performed by personnel addition #1:

Procedure(s) to be performed by personnel addition #2:

Procedure(s) to be performed by personnel addition #3:

III. PROPOSED CHANGES TO THE ORIGINALLY APPROVED PROTOCOL

☐ **A. CHANGE OF SPONSOR/FUNDING SOURCE**

List the new/approved funding source, the project/grant number, the project/grant title (if different from the IACUC protocol title), and the award period.

☐ **B. CHANGE IN PROJECT SITE(S)**

List all new locations (i.e., building and room number(s)) and indicate what procedure(s) will be performed in each new location.

☐ **C. CHANGE OF SPECIES AND/OR STRAIN**

List the species to be added to or removed from the protocol. Please provide the rationale for any species/strain additions and indicate the USDA Pain Category assignment for each new species/strain. Also, list any anticipated changes in veterinary care and/or husbandry due to the new species/strain.

Species and/or strain	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E

☐ **D. CHANGE IN ANIMAL NUMBERS**

List the number of animals to be added to or removed from the protocol. If animals will be added, please provide the justification for the increase and indicate the number of animals to be assigned to each USDA Pain Category.

Species and/or strain	Year (1, 2 or 3)	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E	TOTAL

Will the animals be ordered through CompMed? _____Yes _____ No ***(Identify the source and provide the rationale/justification below.)***

Will the animals require special housing (e.g., specific bedding requirements, isolator cages, special feed, etc.)
_____ Yes ***(Please specify below.)*** _____ No

USDA Pain Code B = *Breeding or holding colony; no animal manipulation.*
USDA Pain Code C = *Procedures involving no or momentary slight pain or distress for which no pain-relieving drugs are used.*
USDA Pain Code D = *Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.*
USDA Pain Code E = *Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.*

X **E. CHANGE IN EXPERIMENTAL PROCEDURES AND/OR INSTRUMENTATION**

Describe, in detail, the proposed change(s). *Please indicate potentially painful/distressing effects on the animals as a result of the proposed change(s). Also, please indicate any expected adverse effects and any changes in monitoring, husbandry, housing, early endpoint, and/or experimental endpoint. Finally, please complete the checklist below.*

Currently, I am approved to perform the experiment as outlined below. I am requesting that the primary means of induction be changed to gas anesthesia and Xylazine be used as a secondary means PRN as determined by [redacted]. Also, I would like to add one extra day of video tape monitoring to include 24hours post infusion. No other parameters of the study will be changed.

Procedure:

[1] Baseline study: Prior to initiation of ketamine-infusion, we will quantify how often and how long the 3 baboons in question display SIB/undesired behavior. Behavior will be monitored twice daily over a 30 minute period daily for 5 consecutive days (Monday-Friday). Animals will serve as their own controls and thus will subsequently be treated with placebo (saline vehicle) or ketamine as described below and behavior monitored and quantified.
[2] Treatment protocol: On the following Monday, baboons will be restrained and sedated with Xylazine (0.5 mg/kg BW, IM) then maintained with Isoflurane (0.5 to 2%) via nose-cone as needed throughout the duration of the procedure. An intravenous catheter will be placed in the antecubital or saphenous vein and a constant infusion of ketamine (0.5mg/kg BW/4 ml physiologic saline/minute) or NaCl alone (4 ml/min) will be administered over a 60 minute period. Physiologic parameters including blood pressure, heart rate and blood oxygenation will be monitored throughout the procedure. After 60 minutes infusion, isoflurane will be stopped and animals returned to their home cage for recovery.
[3] Behavior analysis post ketamine or saline: Starting the same day but at least 4 hours after treatment/placebo, baboons will be observed and SIB/undesired behavior quantified twice daily for 5 days. Observations will be made by the same technician who collected the baseline data; this technician will be blinded as to whether the monkey received ketamine or placebo. The protocol will be repeated the following week but switching placebo/treatment; i.e. baboons serve as their own control.

[redacted] has agreed to assist in analysis and interpretation of the findings and ascertain potential clinical relevance and significance.

Please check all that apply to the proposed change(s). Complete the required IACUC Attachment and submit it (i.e., the original and 17 photocopies) along with the amendment form.	
Biohazardous Agents: ____ Yes __X__ No (e.g., recombinant DNA, RNA, all tissue culture, laboratory-induced infection, cultured pathogens)	Complete and submit **Attachment D**
Radioisotopes, *in vivo*: ____ Yes __X__ No	Complete and submit **Attachment D**
Chemical Agents: ____ Yes _X___ No (e.g., known or suspected chemical hazards, mutagens, or teratogens)	Complete and submit **Attachment D**
Stress or Prolonged Restraint: ____ Yes __X__ No	Complete and submit **Attachment C**
Food and/or Water Deprivation: ____ Yes __X__ No	Complete and submit **Attachment C**
Surgical Procedures: ____ Yes _X___ No (i.e., single survival, multiple survival, or non-survival)	Complete and submit **Attachment E**

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

Antibody Production: ____ Yes _X___ No	Complete and submit **Attachment F**
Toxicity Testing (LD50): ____ Yes _X___ No	Complete and submit **Attachment G**
Lasers or Penetrating Electromagnetic Radiation: ____ Yes _X___ No	
Collection of Tissues, Cells, or Organs: ____ Yes _X___ No (Please explain below.)	
Tumor Transplantation or Induction: ____ Yes __X__ No	
Special Diet: ____ Yes _X___ No (Please explain below.)	
Other : (Please specify below.)	

X **F. CHANGE IN ANESTHESIA, THERAPEUTICS, AND/OR EXPERIMENTAL AGENTS**

	Dose (mg/kg)	Route of Administration	Frequency of Administration
Sedatives/Tranquilizers			
Xylazine	0.5-6mg/kg	IM	As a secondary means of induction PRN
Anesthetics - General			
Isoflurane	0.5 – 2%	Mask	Twice at iv treatment
Anesthetics - Local			

				Length of Administration
Analgesics				
Antibiotics				
Miscellaneous				

EXPERIMENTAL AGENTS:

Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _____

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

Agent: _____ **Agent Vehicle:** _____

Route/Site: _____ **Volume per administration:** _____

Frequency of administration: _____

Expected side effects and/or changes in animal behavior: _____

INVESTIGATOR ASSURANCES:

X	*I understand that it is my responsibility as the Principal Investigator to ensure that all personnel who work on this protocol are adequately trained. That training may be provided by me, an experienced member of the investigative staff, the Attending Veterinarian, a qualified Division of Comparative Medicine (CompMed) staff member, or an appropriate outside source. I hereby affirm that each person added to this protocol by way of this amendment request has been adequately trained to perform the procedure(s) indicated above. Any person who has not been adequately trained will be so trained prior to performing the procedure(s).*
X	*No animal involved in this project will be subjected to discomfort, pain, or distress, unless it is unavoidable in the conduct of scientifically valuable research and approval of such has been approved by the IACUC.*
X	*I agree to comply with all federal and institutional policies governing the use of animals used in this project.*

PRINCIPAL INVESTIGATOR:

Printed Name: Gerald Pepe, Ph.D.

Signature: [signature] **Date:** 5/1/12

APPROVAL SIGNATURES:

PRE-VETERINARY REVIEW: *Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian before the changes are submitted to the IACUC for review.*

Attending Veterinarian Printed Name: [redacted]

Attending Veterinarian Signature: [redacted] **Date:** 5/1/12

FINAL IACUC APPROVAL: *All revisions must be approved by the IACUC prior to implementation.*

IACUC Chair or Vice Chair Printed Name: [redacted]

IACUC Chair or Vice Chair Signature: [redacted] **Date:** 5/21/12

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

OFFICE OF RESEARCH WWW.EVMS.EDU
[redacted] Norfolk, VA 23507 Phone: 757 [redacted] Fax: 757 [redacted]

EVMS
Eastern Virginia Medical School

October 11, 2011

Gerald J. Pepe, Ph.D.
Chair, Department of Physiological Sciences
Eastern Virginia Medical School
[redacted]
Norfolk, Virginia 23507

Dear Dr. Pepe: Jerry

The Institutional Animal Care and Use Committee reviewed your request to amend the protocol entitled, ***Regulation of Fetal-Placental Development in the Primate*** **(IACUC #09-007)**, at its October 6, 2011 meeting. **The following amendments to the protocol were approved:**

1. **permission to perform an intravenous glucose tolerance test (IVGTT) in untreated and treated pregnant animals on day 155-165 of gestation (prior to fetal delivery) to determine whether alteration of fetal/neonatal glucose metabolism and insulin action is a result of and/or associated with a change in maternal carbohydrate homeostasis. It is the understanding of the Committee that a total of nine blood samples @ 3 ml each will be collected over a 60-90 minute period, along with a small volume of blood (0.5 ml) drawn to flush the line and generate a clean sample, and**
2. **the addition of [redacted] to perform Doppler analyses.**

PLEASE NOTE: Although the Division of Comparative Medicine (CompMed) will make every effort to provide housing, husbandry, and veterinary support for your research project, IACUC approval of this amendment request does not guarantee that CompMed will be able to accommodate the work immediately. Available space and resources within the CompMed animal facility are determined by the number of ongoing projects and the animal census at any given time. Please consult with the CompMed management team to confirm availability prior to implementing the approved modification(s).

It is the responsibility of the Principal Investigator to notify the Committee, in writing, of any deviation from the IACUC-approved protocol. Please note that a change in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian prior to submitting the amendment request to the IACUC for approval.

It is also the responsibility of the Principal Investigator to provide for regular observation of the health of the research animals and to provide the Division of Comparative Medicine (CompMed) with the name(s) and telephone number(s) of the person(s) who may be contacted and/or take action in the event that the CompMed staff notes an emergency condition with the research animals.

Thank you for your continued cooperation with the Institutional Animal Care and Use Committee.

Sincerely,

[redacted]

[redacted], *Chair*
Institutional Animal Care and Use Committee

[redacted]

cc: [redacted]
Attending Veterinarian
Division of Comparative Medicine

[redacted]
Program Manager
Division of Comparative Medicine

[redacted]
Associate Dean for Research
Institutional Official

PHYSIOLOGICAL SCIENCES | WWW.EVMS.EDU
P.O. Box 1980 ☐ Norfolk, VA 23501-1980 | Phone: 757 [redacted] ☐ Fax: 757 [redacted]

EVMS
Eastern Virginia Medical School

September 16, 2011

Gerald J. Pepe, PhD
Professor and Chair

[redacted] Chair
Institutional Animal Care and Use Committee
Eastern Virginia Medical School
Norfolk, VA 23507

Dear [redacted]:

I would like to have the appended research protocol (briefly outlined below) amended to my approved IACUC protocol entitled "Regulation of Fetal-Placental Development in the Primate" (#09-007). In the existing protocol, we are approved to treat baboon mothers with estradiol on days 25-59 of gestation or with an aromatase inhibitor (letrozole) or letrozole plus estradiol at other times in gestation and obtain maternal blood samples (2-5 ml) at 1-4 day intervals throughout the pregnancy/study period. Baboon mothers are then allowed to deliver spontaneously at term (day 184) or be delivered by cesarean section at day 165-170 of gestation and the neonate/offspring studied as adults. One of the neonatal studies we are approved to perform is an intravenous glucose tolerance test (IVGTT). Moreover, we recently showed that compared with estrogen replete animals (untreated or treated with letrozole and estradiol), the offspring who developed *in utero* in the relative absence of estradiol (i.e. letrozole-treated *in utero*) exhibit insulin resistance and altered IVGTT as well as elevated insulin levels at birth.

It is critically important that we determine whether alteration of fetal/neonatal glucose metabolism/insulin action is a result of and/or associated with a change in maternal carbohydrate homeostasis. Accordingly, I am requesting permission to perform an IVGTT in our untreated/treated pregnant baboons on day 155-165 of gestation (i.e. prior to fetal delivery) to answer this important question. A maternal IVGTT would utilize our currently approved sampling protocol for IVGTT in offspring and thus involves collection of a total of 9 samples each 3.0 ml over a 60-90 min period, as well as a small volume (0.5 ml) drawn to flush the line and generate a "clean" sample. Moreover, total blood sampling will not exceed protocol guidelines of no more than 8 ml/kg body weight/month or 10% of total blood volume within a two week period. The mother would be receiving a bolus of glucose which should pose no problem to the pregnancy or the fetus; the mother will be monitored for HR and blood pressure and fetal HR will be monitored via non-invasive ultrasound every ten minutes

The IACUC's time and effort in reviewing this amendment are most appreciated.

Sincerely,

Gerald J. Pepe, Ph.D.

RECEIVED
SEP 21 2011
OF RESEARCH
EVMS

REQUEST TO AMEND A PREVIOUSLY APPROVED IACUC PROTOCOL

Institutional Animal Care and Use Committee (IACUC)

Eastern Virginia Medical School
Office of Research

IACUC Office
[redacted]

Office: 757-[redacted]
Fax: 757-[redacted]

FOR OFFICE USE ONLY [redacted]
Date Received: 9/21/11 **Review Method:** X FCR / ___ FR / ___ Administrative (*Personnel Changes Only*)
IBC Approval? X Yes / ___ No **IBC Approval Date:** 9/13/09 **Final Approval Date:** 10/6/11 [redacted]

General Information and Instructions:

1. All requested amendments must be approved by the IACUC **before** they are implemented.
2. Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian **before** the changes are submitted to the IACUC Office.
3. A request to increase the number of animals assigned to a protocol must be approved by the IACUC **before** the animals are ordered.
4. Submit the original signed amendment form, along with seventeen (17) photocopies, to the IACUC Office.
5. ***The IACUC reserves the right to require submission of an IACUC "Initial Review Form" based upon the Committee's assessment of the amendment(s) requested herein.***

I. ADMINISTRATIVE INFORMATION

Protocol Number: 09-007	**Protocol Initial Approval Date: 6/2009** 7/13/09
Protocol Title: Regulation of Fetal – Placental Development in the Primate	
Principal Investigator: Pepe	
Approved USDA Pain Code Level(s) – *not required for personnel additions only*: **B, C and D**	

II. PERSONNEL

List all personnel to be added to or removed from the protocol. ***All persons authorized to work on the protocol must have completed the required CITI and OHSP training modules and must have completed a health/risk assessment with the Occupational Health Department.***

Name	Added (+) or Removed (-)	E-mail Address *(additions only)*	CITI Training Certification Number *(additions only)*	CITI Training Species Module(s) Completed *(additions only)*	OHSP Training Certification Number *(additions only)*	Occupational Health/ Risk Assessment Date *(additions only)*
1. [redacted]	+	[redacted]	[redacted]	Non-human Primate	[redacted]	5/9/11
2.						
3.						

Procedure(s) to be performed by personnel addition #1:
Doppler analysis

Procedure(s) to be performed by personnel addition #2:

Procedure(s) to be performed by personnel addition #3:

III. PROPOSED CHANGES TO THE ORIGINALLY APPROVED PROTOCOL

☐ **A. CHANGE OF SPONSOR/FUNDING SOURCE**

List the new/approved funding source, the project/grant number, the project/grant title (if different from the IACUC protocol title), and the award period.

☐ **B. CHANGE IN PROJECT SITE(S)**

List all new locations (i.e., building and room number(s)) and indicate what procedure(s) will be performed in each new location.

☐ **C. CHANGE OF SPECIES AND/OR STRAIN**

List the species to be added to or removed from the protocol. Please provide the rationale for any species/strain additions and indicate the USDA Pain Category assignment for each new species/strain. Also, list any anticipated changes in veterinary care and/or husbandry due to the new species/strain.

Species and/or strain	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E

☐ **D. CHANGE IN ANIMAL NUMBERS**

List the number of animals to be added to or removed from the protocol. If animals will be added, please provide the justification for the increase and indicate the number of animals to be assigned to each USDA Pain Category.

Species and/or strain	Year (1, 2 or 3)	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E	TOTAL

Will the animals be ordered through CompMed? _____Yes _____ No ***(Identify the source and provide the rationale/justification below.)***

Will the animals require special housing (e.g., specific bedding requirements, isolator cages, special feed, etc.)
_____ Yes ***(Please specify below.)*** _____ No

***USDA Pain Code B** = Breeding or holding colony; no animal manipulation.*
***USDA Pain Code C** = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.*
***USDA Pain Code D** = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.*
***USDA Pain Code E** = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.*

[X] **E. CHANGE IN EXPERIMENTAL PROCEDURES AND/OR INSTRUMENTATION**

Describe, in detail, the proposed change(s). *Please indicate potentially painful/distressing effects on the animals as a result of the proposed change(s). Also, please indicate any expected adverse effects and any changes in monitoring, husbandry, housing, early endpoint, and/or experimental endpoint. Finally, please complete the checklist below.*

Our current approved protocol allows IVGT test in our EVMS colony of juvenile baboons. We propose no change in procedure but request permission to perform the study in our pregnant baboons between gestational age day 155-165. Animals will be monitored as outlined in the approved protocol and fetal HR will be monitored every ten minutes via non-invasive ultrasound. We request to perform this test only once during pregnancy.

Please check all that apply to the proposed change(s). Complete the required IACUC Attachment and submit it (i.e., the original and 17 photocopies) along with the amendment form.	
Biohazardous Agents: ____ **Yes** ____ **No** (e.g., recombinant DNA, RNA, all tissue culture, laboratory-induced infection, cultured pathogens)	Complete and submit **Attachment D**
Radioisotopes, *in vivo*: ____ **Yes** ____ **No**	Complete and submit **Attachment D**
Chemical Agents: ____ **Yes** ____ **No** (e.g., known or suspected chemical hazards, mutagens, or teratogens)	Complete and submit **Attachment D**
Stress or Prolonged Restraint: ____ **Yes** ____ **No**	Complete and submit **Attachment C**
Food and/or Water Deprivation: ____ **Yes** ____ **No**	Complete and submit **Attachment C**
Surgical Procedures: ____ **Yes** ____ **No** (i.e., single survival, multiple survival, or non-survival)	Complete and submit **Attachment E**
Antibody Production: ____ **Yes** ____ **No**	Complete and submit **Attachment F**
Toxicity Testing (LD50): ____ **Yes** ____ **No**	Complete and submit **Attachment G**
Lasers or Penetrating Electromagnetic Radiation: ____ **Yes** ____ **No**	
Collection of Tissues, Cells, or Organs: ____ **Yes** ____ **No** (Please explain below.)	
Tumor Transplantation or Induction: ____ **Yes** ____ **No**	
Special Diet: ____ **Yes** ____ **No** (Please explain below.)	
Other : (Please specify below.)	

***USDA Pain Code B** = Breeding or holding colony; no animal manipulation.*
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***USDA Pain Code D** = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.*
***USDA Pain Code E** = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.*

☐ F. CHANGE IN ANESTHESIA, THERAPEUTICS, AND/OR EXPERIMENTAL AGENTS

	Dose (mg/kg)	Route of Administration	Frequency of Administration	
Sedatives/Tranquilizers				
Anesthetics - General				
Anesthetics - Local				
				Length of Administration
Analgesics				
Antibiotics				
Miscellaneous				

EXPERIMENTAL AGENTS:

Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _____
_____

Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _____
_____

Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____

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USDA Pain Code C = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

INVESTIGATOR ASSURANCES:	
☑	*I understand that it is my responsibility as the Principal Investigator to ensure that all personnel who work on this protocol are adequately trained. That training may be provided by me, an experienced member of the investigative staff, the Attending Veterinarian, a qualified Division of Comparative Medicine (CompMed) staff member, or an appropriate outside source. I hereby affirm that each person added to this protocol by way of this amendment request has been adequately trained to perform the procedure(s) indicated above. Any person who has not been adequately trained will be so trained prior to performing the procedure(s).*
☑	*No animal involved in this project will be subjected to discomfort, pain, or distress, unless it is unavoidable in the conduct of scientifically valuable research and approval of such has been approved by the IACUC.*
☑	*I agree to comply with all federal and institutional policies governing the use of animals used in this project.*

PRINCIPAL INVESTIGATOR:

Printed Name: Gerald J. Pepe Ph.D.	
Signature: Gerald J. Pepe	**Date:** 9/14/11

APPROVAL SIGNATURES:

PRE-VETERINARY REVIEW: *Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian before the changes are submitted to the IACUC for review.*	
Attending Veterinarian Printed Name: [redacted]	
Attending Veterinarian Signature: [redacted]	**Date:** 9/15/11
FINAL IACUC APPROVAL: *All revisions must be approved by the IACUC prior to implementation.*	
IACUC Chair or Vice Chair Printed Name: [redacted]	
IACUC Chair or Vice Chair Signature: [redacted]	**Date:** 10/13/11

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
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USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

IACUC Chair or Vice Chair Signature:	Date:

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

OFFICE OF RESEARCH WWW.EVMS.EDU
[redacted] Norfolk, VA 23507 Phone [redacted] Fax [redacted]

EVMS
Eastern Virginia Medical School

April 9, 2012

Gerald J. Pepe, Ph.D.
Chair, Department of Physiological Sciences
Eastern Virginia Medical School
[redacted]
Norfolk, Virginia 23507

Dear Dr. Pepe: Jerry

The Institutional Animal Care and Use Committee reviewed your request to amend the protocol entitled, ***Regulation of Fetal-placental Development in the Primate*** **(IACUC #09-007)**, at its April 5, 2012 meeting. **The following amendments to the protocol were approved:**

1. **permission to administer Ketamine to test its effect on alleviating or minimizing self-injurious behavior (SIB) and/or other undesired behavior. It is the understanding of the Committee that a double-blinded study will be performed using three baboons from the current colony. The Committee further understands that a continuous infusion of Ketamine will be administered for 60 minutes @ a dose of 0.5 mg/kg body weight.**
2. **permission to administer Xylazine as a sedative prior to administering the anesthetic agent. It is the understanding of the Committee that Xylazine will be administered intramuscularly at a dose of 0.5-6.0 mg/kg.**
3. **permission to administer Isoflurane as an anesthetic agent. It is the understanding of the Committee that Isoflurane will be administered twice via inhalation at a concentration of 0.5-2%.**

Although the Division of Comparative Medicine (CompMed) will make every effort to provide housing, husbandry, and veterinary support for your research project, IACUC approval of this amendment request does not guarantee that CompMed will be able to accommodate the work immediately. Available space and resources within the CompMed animal facility are determined by the number of ongoing projects and the animal census at any given time. Please consult with the CompMed management team to confirm availability prior to implementing the approved modification(s).

It is the responsibility of the Principal Investigator to notify the Committee, in writing, of any deviation from the IACUC-approved protocol. Please note that a change in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian prior to submitting the amendment request to the IACUC for approval.

It is also the responsibility of the Principal Investigator to provide for regular observation of the health of the research animals and to provide the Division of Comparative Medicine (CompMed) with the name(s) and telephone number(s) of the person(s) who may be contacted and/or take action in the event that the CompMed staff notes an emergency condition with the research animals.

Thank you for your continued cooperation with the Institutional Animal Care and Use Committee.

Sincerely,

[redacted]

[redacted] *Chair*
Institutional Animal Care and Use Committee

[redacted]

cc: [redacted]
Attending Veterinarian
Division of Comparative Medicine

[redacted]
Program Manager
Division of Comparative Medicine

[redacted]
Associate Dean for Research
Institutional Official

April 2010
Updated February 2011, March 2011

RECEIVED
MAR 8 2012

REQUEST TO AMEND A PREVIOUSLY APPROVED IACUC PROTOCOL

Institutional Animal Care and Use Committee (IACUC)

Eastern Virginia Medical School
Office of Research

IACUC Office
[redacted]

Office: 757-[redacted]
Fax: 757-[redacted]

FOR OFFICE USE ONLY [redacted]
Date Received: 3/7/12 **Review Method:** X FCR / ____ FR / ____ Administrative (*Personnel Changes Only*)
IBC Approval? X Yes / ____ No **IBC Approval Date:** 9/12/09 **Final Approval Date:** 4/5/12 [redacted]

General Information and Instructions:

1. All requested amendments must be approved by the IACUC before they are implemented.
2. Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian before the changes are submitted to the IACUC Office.
3. A request to increase the number of animals assigned to a protocol must be approved by the IACUC before the animals are ordered.
4. Submit the original signed amendment form, along with seventeen (17) photocopies, to the IACUC Office.
5. *The IACUC reserves the right to require submission of an IACUC "Initial Review Form" based upon the Committee's assessment of the amendment(s) requested herein.*

I. ADMINISTRATIVE INFORMATION

Protocol Number: 09-007	**Protocol Initial Approval Date:** July 13, 2009
Protocol Title: Regulation of Fetal-Placental Development in the Primate	
Principal Investigator: Gerald J. Pepe, Ph.D.	
Approved USDA Pain Code Level(s) – *not required for personnel additions only*: B, C, and D	

II. PERSONNEL

List all personnel to be added to or removed from the protocol. All persons authorized to work on the protocol must have completed the required CITI/LATA and OHSP training modules and must have completed a health/risk assessment with the Occupational Health Department.

Name	Added (+) or Removed (-)	E-mail Address *(additions only)*	CITI/LATA Training Certification Number *(additions only)*	CITI/LATA Training Species Module(s) Completed *(additions only)*	OHSP Training Certification Number *(additions only)*	Occupational Health/ Risk Assessment Date *(additions only)*
1.						
2.						
3.						

Procedure(s) to be performed by personnel addition #1:

Procedure(s) to be performed by personnel addition #2:

Procedure(s) to be performed by personnel addition #3:

III. PROPOSED CHANGES TO THE ORIGINALLY APPROVED PROTOCOL

☐ **A. CHANGE OF SPONSOR/FUNDING SOURCE**

List the new/approved funding source, the project/grant number, the project/grant title (if different from the IACUC protocol title), and the award period.

☐ **B. CHANGE IN PROJECT SITE(S)**

List all new locations (i.e., building and room number(s)) and indicate what procedure(s) will be performed in each new location.

☐ **C. CHANGE OF SPECIES AND/OR STRAIN**

List the species to be added to or removed from the protocol. Please provide the rationale for any species/strain additions and indicate the USDA Pain Category assignment for each new species/strain. Also, list any anticipated changes in veterinary care and/or husbandry due to the new species/strain.

Species and/or strain	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E

☐ **D. CHANGE IN ANIMAL NUMBERS**

List the number of animals to be added to or removed from the protocol. If animals will be added, please provide the justification for the increase and indicate the number of animals to be assigned to each USDA Pain Category.

Species and/or strain	Year (1, 2 or 3)	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E	TOTAL

Will the animals be ordered through CompMed? _____Yes _____ No ***(Identify the source and provide the rationale/justification below.)***

Will the animals require special housing (e.g., specific bedding requirements, isolator cages, special feed, etc.)
_____ Yes ***(Please specify below.)*** _____ No

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
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USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

X **E. CHANGE IN EXPERIMENTAL PROCEDURES AND/OR INSTRUMENTATION**

Describe, in detail, the proposed change(s). *Please indicate potentially painful/distressing effects on the animals as a result of the proposed change(s). Also, please indicate any expected adverse effects and any changes in monitoring, husbandry, housing, early endpoint, and/or experimental endpoint. Finally, please complete the checklist below.*

Recent reports on national media and scientific evidence support the concept that intravenous ketamine acting via glutaminergic mechanisms rapidly alleviates depression and other manic-depressive conditions in patients not helped by or responsive to the plethora of psycho active drugs which target serotonergic mechanisms. [redacted], DVM, attending veterinarian at EVMS suggested that it would be important to ascertain whether intravenous ketamine would alleviate or at least minimize self-injurious behavior (SIB) and/or other undesired behavior (e.g. excessive grooming; repetitive circling movements) that sometime become manifest in nonhuman primates. Although our laboratory has over the years only seen SIB in a small number of baboons housed in our facility, [redacted] still feels it would be important to perform a double-blinded study on the 3 baboons currently in our colony that show excessive movements/grooming and in one case SIB and thus provide insight into approaches to alleviate the problem now and in the future.

[redacted], myself and [redacted] also met with and discussed this potential study with [redacted]., Chairman of the Department of Psychiatry and Behavioral Sciences at EVMS. [redacted] enthusiastically endorsed such an experiment and felt that this relatively straight-forward study could provide substantive new findings that could translate to and thus help humans suffering from depressive behaviors and not responsive to therapy/drugs. Indeed, the primary target of almost all drugs for depression/behavior disorders is serotonin. Moreover, [redacted] also felt that results could apply to people, particularly young adults and children suffering from conditions such as Autism and schizophrenia and in whom undesired behaviors including self-injury are often manifest and potentially life-threatening.

Currently, we have identified 3 baboons, # I096(Niki), #M181(Molly), and #G194(Patty) that have developed and now show unwanted behaviors including over-grooming, repetitive circling and in one animal SIB. These undesired behaviors have compromised the ability to employ these animals in research projects as well as animal well being. Based on current literature, and recent case reports, a constant intravenous infusion of ketamine 0.5mg/min/kg body weight for 60 minutes dramatically changed the mood of depressed humans for a period of up-to one week. We therefore will employ this dose as a starting point for our experimental protocol outlined below.

Procedure:

[1] Baseline study: Prior to initiation of ketamine-infusion, we will quantify how often and how long the 3 baboons in question display SIB/undesired behavior. Behavior will be monitored twice daily over a 30 minute period daily for 5 consecutive days (Monday-Friday). Animals will serve as their own controls and thus will subsequently be treated with placebo (saline vehicle) or ketamine as described below and behavior monitored and quantified.
[2] Treatment protocol: On the following Monday, baboons will be restrained and sedated with Xylazine (0.5 mg/kg BW, IM) then maintained with Isoflurane (0.5 to 2%) via nose-cone as needed throughout the duration of the procedure. An intravenous catheter will be placed in the antecubital or saphenous vein and a constant infusion of ketamine (0.5mg/kg BW/4 ml physiologic saline/minute) or NaCl alone (4 ml/min) will be administered over a 60 minute period. Physiologic parameters including blood pressure, heart rate and blood oxygenation will be monitored throughout the procedure. After 60 minutes infusion, isoflurane will be stopped and animals returned to their home cage for recovery.
[3] Behavior analysis post ketamine or saline: Starting the same day but at least 4 hours after treatment/placebo, baboons will be observed and SIB/undesired behavior quantified twice daily for 5 days. Observations will be made by the same technician who collected the baseline data; this technician will be blinded as to whether the monkey received ketamine or placebo. The protocol will be repeated the following week but switching placebo/treatment; i.e. baboons serve as their own control.

[redacted] has agreed to assist in analysis and interpretation of the findings and ascertain potential clinical relevance and significance.

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USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

Please check all that apply to the proposed change(s). Complete the required IACUC Attachment and submit it (i.e., the original and 17 photocopies) along with the amendment form.	
Biohazardous Agents: ____ **Yes** __X__ **No** (e.g., recombinant DNA, RNA, all tissue culture, laboratory-induced infection, cultured pathogens)	Complete and submit **Attachment D**
Radioisotopes, *in vivo*: ____ **Yes** __X__ **No**	Complete and submit **Attachment D**
Chemical Agents: ____ **Yes** _X___ **No** (e.g., known or suspected chemical hazards, mutagens, or teratogens)	Complete and submit **Attachment D**
Stress or Prolonged Restraint: ____ **Yes** __X__ **No**	Complete and submit **Attachment C**
Food and/or Water Deprivation: ____ **Yes** __X__ **No**	Complete and submit **Attachment C**
Surgical Procedures: ____ **Yes** _X___ **No** (i.e., single survival, multiple survival, or non-survival)	Complete and submit **Attachment E**
Antibody Production: ____ **Yes** _X___ **No**	Complete and submit **Attachment F**
Toxicity Testing (LD50): ____ **Yes** _X___ **No**	Complete and submit **Attachment G**
Lasers or Penetrating Electromagnetic Radiation: ____ **Yes** _X___ **No**	
Collection of Tissues, Cells, or Organs: ____ **Yes** _X___ **No** (Please explain below.)	
Tumor Transplantation or Induction: ____ **Yes** __X__ **No**	
Special Diet: ____ **Yes** _X___ **No** (Please explain below.)	
Other : (Please specify below.)	

[X] **F. CHANGE IN ANESTHESIA, THERAPEUTICS, AND/OR EXPERIMENTAL AGENTS**

	Dose (mg/kg)	Route of Administration	Frequency of Administration	
Sedatives/Tranquilizers				
Xylazine	0,5-6mg/kg	IM	Prior to anesthesia	
Anesthetics - General				
Isoflurane	0.5 – 2%	Mask	Twice at iv treatment	
Anesthetics - Local				
				Length of Administration

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

Analgesics				
Antibiotics				
Miscellaneous				

EXPERIMENTAL AGENTS:

Agent: Ketamine **Agent Vehicle:** 0.9 % NaCl solution
Route/Site: IV **Volume per administration:** (0.5mg/kg) in 250ml
Frequency of administration: once
Expected side effects and/or changes in animal behavior: Animals may stop adverse behavior

Agent: Placebo **Agent Vehicle:** 0.9 % NaCl solution
Route/Site: IV **Volume per administration:** 250 ml
Frequency of administration: Once
Expected side effects and/or changes in animal behavior: None

Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _____

INVESTIGATOR ASSURANCES:

X	*I understand that it is my responsibility as the Principal Investigator to ensure that all personnel who work on this protocol are adequately trained. That training may be provided by me, an experienced member of the investigative staff, the Attending Veterinarian, a qualified Division of Comparative Medicine (CompMed) staff member, or an appropriate outside source. I hereby affirm that each person added to this protocol by way of this amendment request has been adequately trained to perform the procedure(s) indicated above. Any person who has not been adequately trained will be so trained prior to performing the procedure(s).*
X	*No animal involved in this project will be subjected to discomfort, pain, or distress, unless it is unavoidable in the conduct of scientifically valuable research and approval of such has been approved by the IACUC.*
X	*I agree to comply with all federal and institutional policies governing the use of animals used in this project.*

***USDA Pain Code B** = Breeding or holding colony; no animal manipulation.*
***USDA Pain Code C** = Procedures involving no or momentary slight pain or distress for which no pain-relieving drugs are used.*
***USDA Pain Code D** = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.*
***USDA Pain Code E** = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.*

PRINCIPAL INVESTIGATOR:	
Printed Name: Gerald Pepe, Ph.D.	
Signature:	Date: 3/8/12

APPROVAL SIGNATURES:	
PRE-VETERINARY REVIEW: *Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian before the changes are submitted to the IACUC for review.* **Attending Veterinarian Printed Name:** [redacted] **Attending Veterinarian Signature:** [redacted]	Date: 3/8/12
FINAL IACUC APPROVAL: *All revisions must be approved by the IACUC prior to implementation.* **IACUC Chair or Vice Chair Printed Name:** [redacted] **IACUC Chair or Vice Chair Signature:** [redacted]	Date: 4/16/12

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

OFFICE OF RESEARCH | WWW.EVMS.EDU
[redacted] Norfolk, VA 23501 | Phone: 757[redacted] | Fax: 757[redacted]

EVMS
Eastern Virginia Medical School

October 11, 2011

Gerald J. Pepe, Ph.D.
Chair, Department of Physiological Sciences
Eastern Virginia Medical School
[redacted]
Norfolk, Virginia 23507

Dear Dr. Pepe: Jerry

The Institutional Animal Care and Use Committee reviewed your amendment request (*i.e., permission to perform non-survival surgery in the EVMS-reared animals that have completed the juvenile study*) to the protocol entitled, ***Regulation of Fetal-Placental Development in the Primate*** **(IACUC #09-007)**, at its October 6, 2011 meeting. **The amendment to the protocol was approved.**

> ***NOTE:*** ***It is the understanding of the Committee that skeletal muscle, intra-abdominal fat, subcutaneous fat, and the pancreas will be harvested to determine the site(s) and mechanisms of estrogen in estrogen-replete animals vs. animals that developed in the absence of estrogen in utero. The Committee further understands that at least three biopsies of the tail region of the pancreas will be taken.***

PLEASE NOTE: Although the Division of Comparative Medicine (CompMed) will make every effort to provide housing, husbandry, and veterinary support for your research project, IACUC approval of this amendment request does not guarantee that CompMed will be able to accommodate the work immediately. Available space and resources within the CompMed animal facility are determined by the number of ongoing projects and the animal census at any given time. Please consult with the CompMed management team to confirm availability prior to implementing the approved modification(s).

It is the responsibility of the Principal Investigator to notify the Committee, in writing, of any deviation from the IACUC-approved protocol. Please note that a change in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian prior to submitting the amendment request to the IACUC for approval.

It is also the responsibility of the Principal Investigator to provide for regular observation of the health of the research animals and to provide the Division of Comparative Medicine (CompMed) with the name(s) and telephone number(s) of the person(s) who may be contacted and/or take action in the event that the CompMed staff notes an emergency condition with the research animals.

Thank you for your continued cooperation with the Institutional Animal Care and Use Committee.

Sincerely,

[redacted]

[redacted] *Chair*
Institutional Animal Care and Use Committee

[redacted]

cc: [redacted]
Attending Veterinarian
Division of Comparative Medicine

[redacted]
Program Manager
Division of Comparative Medicine

[redacted]
Associate Dean for Research
Institutional Official

PHYSIOLOGICAL SCIENCES WWW.EVMS.EDU
P.O. Box 1980 Norfolk, VA 23501-1980 Phone: 757.[redacted] Fax: 757[redacted]

EVMS
Eastern Virginia Medical School

Gerald J. Pepe, PhD
Professor and Chair

September 8, 2011

[redacted] Chair
Institutional Animal Care and Use Committee
Eastern Virginia Medical School
Norfolk, VA 23507

Dear [redacted]

I would like to have the appended research protocol (briefly outlined below) amended to my approved IACUC protocol entitled "Regulation of Fetal-Placental Development in the Primate" (#09-007). In the existing protocol, we are approved to allow baboon mothers treated with estradiol on days 25-59 of gestation or with an aromatase inhibitor (letrozole) at other times in gestation to deliver spontaneously at term (day 184) or be delivered by cesarean section at day 165 of gestation and study the neonate into adulthood (e.g. growth; glucose tolerance; ovarian/reproductive function; adrenal function) to test the hypothesis that estrogen *in utero* programs physiologic function in adulthood. Currently, we have a colony of 18 offspring born to mothers treated *in utero* with nothing (control), letrozole or letrozole and estradiol. In this colony of animals, we have recently shown that compared with estrogen replete animals (untreated or treated with letrozole and estradiol), the offspring who developed *in utero* in the relative absence of estradiol (i.e. letrozole-treated *in utero*) exhibited a delay in onset of puberty, alterations in menstrual cycle length and peripheral blood levels of gonadotropins and steroid hormones as well as levels of adrenal hormones. In addition, these estrogen-deprived offspring exhibit insulin resistance and altered glucose tolerance tests.

Clearly, we view this colony of offspring as an invaluable resource. However, we have essentially completed the studies for which we are currently approved in several offspring. Accordingly I am requesting that these animals be euthanized as additional animals will become part of this colony starting in the next few months and continuing over the next few years. Additional animals are required to enhance our studies of estrogen programming.

As we go forward, however, we do not plan to simply repeat studies but rather build upon our current findings and move science forward and not laterally. As mentioned above, compared with estrogen-replete baboons (e.g. control animals), offspring who developed in the absence of estrogen *in utero* exhibit high insulin levels and abnormal glucose tolerance as well as insulin resistance. We are in a position to begin to determine the site(s) (e.g. skeletal muscle; adipose tissue, the pancreatic beta cell) and mechanisms (insulin receptor function/action; insulin secretion; glucose transporter function/expression) of this action of estrogen. As a necessary first step, it is essential that we collect biopsies of skeletal muscle, abdominal fat/adipose tissue and

pancreas and confirm that site of collection, specimen size/weight are uniform and provide sufficient sample for multiple analyses, e.g., immunohistochemistry, RT-PCR. Therefore we would also like to amend the protocol to include a non-survival surgery to collect under isoflurane anesthesia samples of skeletal muscle (gluteus), intra-abdominal fat, and subcutaneous fat. In addition, using a soft-tissue needle, we also request collection of at least 3 biopsies of the tail region of the pancreas. At the conclusion of these collections, the animals will be euthanized per protocol and tissues including the pancreas harvested as per our existing protocol.

The IACUC's time and effort in reviewing this amendment are most appreciated.

Sincerely,

Gerald J. Pepe, Ph.D.

GJP[redacted]

April 2010
Updated February 2011

RECEIVED
SEP 12 2011
OFFICE OF RESEARCH EVMS

REQUEST TO AMEND A PREVIOUSLY APPROVED IACUC PROTOCOL

Institutional Animal Care and Use Committee (IACUC)

Eastern Virginia Medical School
Office of Research

IACUC Office
[redacted]

Office: 757-[redacted]
Fax: 757-[redacted]

FOR OFFICE USE ONLY

Date Received: 9/12/11 [redacted] **Review Method:** 1 FCR / ____ FR / ____ Administrative (*Personnel Changes Only*)

IBC Approval? 1 Yes / ____ No **IBC Approval Date:** 7/12/09 **Final Approval Date:** 10/6/11 [redacted]

General Information and Instructions:

1. All requested amendments must be approved by the IACUC **before** they are implemented.
2. Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian **before** the changes are submitted to the IACUC Office.
3. A request to increase the number of animals assigned to a protocol must be approved by the IACUC **before** the animals are ordered.
4. Submit the original signed amendment form, along with seventeen (17) photocopies, to the IACUC Office.
5. ***The IACUC reserves the right to require submission of an IACUC "Initial Review Form" based upon the Committee's assessment of the amendment(s) requested herein.***

I. ADMINISTRATIVE INFORMATION

Protocol Number: 09-007	**Protocol Initial Approval Date:** ~~06/2009~~ 7/13/09
Protocol Title: Regulation of Fetal-Placental Development in the Primate	
Principal Investigator: Gerald J Pepe	
Approved USDA Pain Code Level(s) – *not required for personnel additions only*: **B, C, and D**	

II. PERSONNEL

List all personnel to be added to or removed from the protocol. ***All persons authorized to work on the protocol must have completed the required CITI and OHSP training modules and must have completed a health/risk assessment with the Occupational Health Department.***

Name	Added (+) or Removed (-)	E-mail Address *(additions only)*	CITI Training Certification Number *(additions only)*	CITI Training Species Module(s) Completed *(additions only)*	OHSP Training Certification Number *(additions only)*	Occupational Health/Risk Assessment Date *(additions only)*

Procedure(s) to be performed by personnel addition #1:

Procedure(s) to be performed by personnel addition #2:

Procedure(s) to be performed by personnel addition #3:

III. PROPOSED CHANGES TO THE ORIGINALLY APPROVED PROTOCOL

☐ **A. CHANGE OF SPONSOR/FUNDING SOURCE**

List the new/approved funding source, the project/grant number, the project/grant title (if different from the IACUC protocol title), and the award period.

☐ **B. CHANGE IN PROJECT SITE(S)**

List all new locations (i.e., building and room number(s)) and indicate what procedure(s) will be performed in each new location.

☐ **C. CHANGE OF SPECIES AND/OR STRAIN**

List the species to be added to or removed from the protocol. Please provide the rationale for any species/strain additions and indicate the USDA Pain Category assignment for each new species/strain. Also, list any anticipated changes in veterinary care and/or husbandry due to the new species/strain.

Species and/or strain	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E

[X] **E. CHANGE IN EXPERIMENTAL PROCEDURES AND/OR INSTRUMENTATION**

Describe, in detail, the proposed change(s). *Please indicate potentially painful/distressing effects on the animals as a result of the proposed change(s). Also, please indicate any expected adverse effects and any changes in monitoring, husbandry, housing, early endpoint, and/or experimental endpoint. Finally, please complete the checklist below.*

In study three (3) of our current protocol #09-007, pregnant baboons are treated with nothing or Letrozole or Letrozole plus Estradiol daily on days 100-165 of gestation, animals then delivered via cesarean section at day 165 of gestation (term = day 184) or allowed to spontaneously deliver at term and raise the neonate. As a terminal procedure for those now adult EVMS reared baboons that have completed the already approved juvenile study, we propose the following: Baboons are sedated with ketamine (10mg/kg im) and prepped/monitored for the procedure under Comp Med SOP. A baseline blood sample (5ml) is obtained from the saphenous vein using a 21g needle to determine blood chemistries (Na, K, etc), gases (pCO2, pO2) and acid/base status and subsequent analysis of estradiol, progesterone and androgens. Heart rate, blood pressure, respiration rate, oxygen saturation and body temp are monitored by Comp Med throughout the procedure; at surgical plane of anesthesia is maintained. A 2-3cm incision is made in the bicep to expose the muscle. A section of the muscle will be removed using either a surgical blade or dissecting scissors. The incision will be secured with a running stitch. A second incision will be made from the xiphoid process to the pubis. A section of abdominal and subcutaneous fat will be excised. Finally, the pancreas is isolated using blunt dissection. One biopsy is taken from the tail region using a Ross Modified Silverman needle (2.3mm x 85m). The pancreas is then completely removed for further sampling. The surrounding vessels are clamped immediately to control bleeding. As soon as the pancreas is free, Fatal Plus (Pentobarbital Solution) euthanasia solution is administered (1ml/4.5kg/bw) IV followed by a saline flush. Euthanasia is confirmed by the absence of HB and flat ECG. Subsequent administration of FP will follow PRN until death is confirmed.

Please check all that apply to the proposed change(s). Complete the required IACUC Attachment and submit it (i.e., the original and 17 photocopies) along with the amendment form.	
Biohazardous Agents: ____ **Yes** ____ **No** (e.g., recombinant DNA, RNA, all tissue culture, laboratory-induced infection, cultured pathogens)	Complete and submit **Attachment D**
Radioisotopes, *in vivo*: ____ **Yes** ____ **No**	Complete and submit **Attachment D**
Chemical Agents: ____ **Yes** ____ **No** (e.g., known or suspected chemical hazards, mutagens, or teratogens)	Complete and submit **Attachment D**
Stress or Prolonged Restraint: ____ **Yes** ____ **No**	Complete and submit **Attachment C**
Food and/or Water Deprivation: ____ **Yes** ____ **No**	Complete and submit **Attachment C**
Surgical Procedures: ____ **Yes** ____ **No** (i.e., single survival, multiple survival, or non-survival)	Complete and submit **Attachment E**
Antibody Production: ____ **Yes** ____ **No**	Complete and submit **Attachment F**
Toxicity Testing (LD50): ____ **Yes** ____ **No**	Complete and submit **Attachment G**
Lasers or Penetrating Electromagnetic Radiation: __ **Yes** __ **No**	
Collection of Tissues, Cells, or Organs: ____ **Yes** ____ **No** (Please explain below.)	
Tumor Transplantation or Induction: ____ **Yes** ____ **No**	
Special Diet: ____ **Yes** ____ **No** (Please explain below.)	
Other : (Please specify below.)	

X **F. CHANGE IN ANESTHESIA, THERAPEUTICS, AND/OR EXPERIMENTAL AGENTS**

	Dose (mg/kg)	Route of Administration	Frequency of Administration	
Sedatives/Tranquilizers				
Ketamine	10-15mg/kg	IM	chemical restraint for serotonin challenge	
Anesthetics - General				
Isoflurane	~2 %MAC	Inhalant	At time of procedure challenge	
Anesthetics - Local				
Analgesics				
Antibiotics				
Miscellaneous				
Fatal Plus Euthanasia solution	1ml/4.5kg	IV	At completion of procedure for termination	
Fatal Plus Euthanasia solution	0.5ml/4.5kg	Iv	PRN	

EXPERIMENTAL AGENTS:

Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _

Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:**
Frequency of administration:
Expected side effects and/or changes in animal behavior:

Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _____

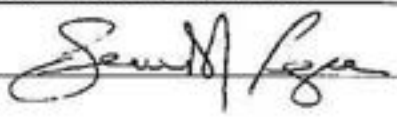
INVESTIGATOR ASSURANCES:

☑	*I understand that it is my responsibility as the Principal Investigator to ensure that all personnel who work on this protocol are adequately trained. That training may be provided by me, an experienced member of the investigative staff, the Attending Veterinarian, a qualified Division of Comparative Medicine (CompMed) staff member, or an appropriate outside source. I hereby affirm that each person added to this protocol by way of this amendment request has been adequately trained to perform the procedure(s) indicated above. Any person who has not been adequately trained will be so trained prior to performing the procedure(s).*
☑	*No animal involved in this project will be subjected to discomfort, pain, or distress, unless it is unavoidable in the conduct of scientifically valuable research and approval of such has been*

	adequately trained to perform the procedure(s) indicated above. Any person who has not been adequately trained will be so trained prior to performing the procedure(s).
☑	*No animal involved in this project will be subjected to discomfort, pain, or distress, unless it is unavoidable in the conduct of scientifically valuable research and approval of such has been approved by the IACUC.*
☑	*I agree to comply with all federal and institutional policies governing the use of animals used in this project.*

PRINCIPAL INVESTIGATOR:

Printed Name: Gerald J. Pepe, Ph.D.

Signature:  **Date:** 9-8-11

APPROVAL SIGNATURES:

PRE-VETERINARY REVIEW: *Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian before the changes are submitted to the IACUC for review.*

Attending Veterinarian Printed Name: [redacted]

Attending Veterinarian Signature: [redacted] **Date:** 9-8-11

FINAL IACUC APPROVAL: *All revisions must be approved by the IACUC prior to implementation.*

IACUC Chair or Vice Chair Printed Name: [redacted]

IACUC Chair or Vice Chair Signature: [redacted] **Date:** 10/13/11

OFFICE OF RESEARCH WWW.EVMS.EDU
[redacted] Norfolk, VA 23501 Phone: 757[redacted] Fax: 757[redacted]

EVMS
Eastern Virginia Medical School

March 24, 2010

Gerald J. Pepe, Ph.D.
Dean and Provost
Department of Physiological Sciences
Eastern Virginia Medical School
[redacted]
Norfolk, Virginia 23507

Dear Dr. Pepe: Jerry

The Institutional Animal Care and Use Committee (IACUC) has reviewed the requested modification regarding your request to amend the protocol entitled, ***Regulation of Fetal-Placental Development in the Primate*** **(IACUC #09-007).** Your original request was reviewed by the IACUC at its March 4, 2010 meeting. **The amendment (*i.e., permission to test the pituitary-adrenal axis in 7+ year old animals with adrenal glands twice the normal size which secrete excess androgens in utero by testing their response to ACTH and Betamethasone/ACTH*) has been approved.**

It is the responsibility of the Principal Investigator to notify the Committee, in writing, of any deviation from the IACUC-approved protocol. Please note that a change in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian prior to submitting the amendment request to the IACUC for approval.

It is also the responsibility of the Principal Investigator to provide for regular observation of the health of research animals and to provide the Division of Comparative Medicine (CompMed) with the name(s) and telephone number(s) of the person(s) who may be contacted and/or take action in the event that the CompMed staff notes an emergency condition with the research animals.

Thank you for your continued cooperation with the Institutional Animal Care and Use Committee.

Sincerely,

[redacted]

[redacted] *Chair*
Institutional Animal Care and Use Committee

[redacted]

cc: [redacted]
Interim Department Chair

[redacted]
Interim Attending Veterinarian
Division of Comparative Medicine

[redacted]
Project Manager
Division of Comparative Medicine

[redacted]
Associate Dean for Research
Institutional Official

OFFICE OF RESEARCH [redacted] Norfolk, VA 23507 | Phone: 757 [redacted] Fax: 75[redacted] WWW.EVMS.EDU

EVMS
Eastern Virginia Medical School

May 4, 2012

Gerald J. Pepe, Ph.D.
Chair, Department of Physiological Sciences
Eastern Virginia Medical School
[redacted]
Norfolk, Virginia 23507

Dear Dr. Pepe:

The Institutional Animal Care and Use Committee reviewed your request to amend the protocol entitled, ***Regulation of Fetal-placental Development in the Primate*** **(IACUC #09-007)**, at its May 3, 2012 meeting. **The following amendments to the protocol were approved:**

1. **permission to extend the gestation age to include days 80-165, and**
2. **permission to perform two maternal IVGTT tests between days 80 and 165 to ascertain whether values for maternal IVGTT at term reflect a developmental change or are truly the result of treatment. It is the understanding of the Committee that the first test will be performed between days 80-120 of gestation, which is earlier than currently approved, and the second test will be performed between days 155-165 as currently approved. The Committee further understands that there will be a minimum of 4 weeks between IVGTT tests.**

Although the Division of Comparative Medicine (CompMed) will make every effort to provide housing, husbandry, and veterinary support for your research project, IACUC approval of this amendment request does not guarantee that CompMed will be able to accommodate the work immediately. Available space and resources within the CompMed animal facility are determined by the number of ongoing projects and the animal census at any given time. Please consult with the CompMed management team to confirm availability prior to implementing the approved modification(s).

It is the responsibility of the Principal Investigator to notify the Committee, in writing, of any deviation from the IACUC-approved protocol. Please note that a change in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian prior to submitting the amendment request to the IACUC for approval.

It is also the responsibility of the Principal Investigator to provide for regular observation of the health of the research animals and to provide the Division of Comparative Medicine (CompMed) with the name(s) and telephone number(s) of the person(s) who may be contacted and/or take action in the event that the CompMed staff notes an emergency condition with the research animals.

Thank you for your continued cooperation with the Institutional Animal Care and Use Committee.

Sincerely,

[redacted]

[redacted] *Chair*
Institutional Animal Care and Use Committee

[redacted]

cc: [redacted]
Attending Veterinarian
Division of Comparative Medicine

[redacted]
Program Manager
Division of Comparative Medicine

[redacted]
Associate Dean for Research
Institutional Official

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P.O. Box 1980 ▫ Norfolk, VA 23501-1980 | Phone: 757 [redacted] ▫ Fax: 757 [redacted]

EVMS
Eastern Virginia Medical School

April 18, 2012

Gerald J. Pepe, PhD
Professor and Chair

[redacted]., Chair
Institutional Animal Care and Use Committee
Eastern Virginia Medical School
Norfolk, VA 23507

Dear [redacted]:

I would like to have the appended research protocol (briefly outlined below) amended to my approved IACUC protocol entitled "Regulation of Fetal-Placental Development in the Primate" (#09-007). In this protocol, we are approved to treat baboon mothers with estradiol on days 25-59 of gestation or with the aromatase inhibitor letrozole or letrozole plus estradiol at other times in gestation and obtain maternal blood samples (2-5 ml) at 1-4 day intervals during the study period. Baboon mothers are then allowed to deliver spontaneously at term (day 184) or be delivered by cesarean section at day 165-170 of gestation and the neonate studied as adults. One of the neonatal studies approved is an intravenous glucose tolerance test (IVGTT).

In September, 2012, I submitted and the IACUC approved an amendment permitting our laboratory to perform a maternal IVGTT in treated/untreated animals on day 155-165 of gestation (i.e. prior to fetal delivery) to determine whether any alteration of fetal/neonatal glucose metabolism reflects a change in maternal carbohydrate homeostasis. It has become apparent to us that to ascertain whether values for maternal IVGTT at term reflect a developmental change or are truly the result of treatment, we need to perform an additional IVGTT earlier in the pregnancy. Accordingly, I am requesting that we be permitted to perform two maternal IVGTTs, one between days 80-120 of gestation and the second as currently approved, i.e. at days 155-165 of gestation. Both maternal IVGTT would utilize our currently approved sampling protocol i.e., collection of a total of 9 maternal blood samples each 3.0 ml over a 60-90 min period, as well as a small volume (0.5 ml) drawn to flush the line and generate a "clean" sample. Moreover, total blood sampling will not exceed protocol guidelines of no more than 8 ml/kg body weight/month or 10% of total blood volume within a two week period. The mother would be receiving a bolus of glucose which should pose no problem to the pregnancy or the fetus; the mother will be monitored for HR and blood pressure and fetal HR will be monitored via non-invasive ultrasound every ten minutes

The IACUC's time and effort in reviewing this amendment are most appreciated.

Sincerely,

Gerald J. Pepe, Ph.D.

April 2010
Updated February 2011; March 2011; April 2012

RECEIVED
APR 19 2012
OFFICE USE

REQUEST TO AMEND A PREVIOUSLY APPROVED IACUC PROTOCOL

Institutional Animal Care and Use Committee (IACUC)

Eastern Virginia Medical School
Office of Research

IACUC Office
[redacted]

Office: 757-[redacted]
Fax: 757-[redacted]

FOR OFFICE USE ONLY
Date Received: 4/19/12 [redacted] **Review Method:** X FCR / ___ FR / ___ Administrative (*Personnel Changes Only*)
IBC Approval? X Yes / ___ No **IBC Approval Date:** 4/4/09 **Final Approval Date:** 5/3/12 [redacted]

General Information and Instructions:

1. All requested amendments must be approved by the IACUC **before** they are implemented.
2. Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian **before** the changes are submitted to the IACUC Office.
3. A request to increase the number of animals assigned to a protocol must be approved by the IACUC **before** the animals are ordered.
4. **Submit the original signed amendment form to the IACUC Office located in [redacted], and e-mail the MSWord version of the form to the IACUC Administrator.**
5. ***The IACUC reserves the right to require submission of an IACUC "Initial Review Form" based upon the Committee's assessment of the amendment(s) requested herein.***

I. ADMINISTRATIVE INFORMATION

Protocol Number: 09-007	**Protocol Initial Approval Date: 7/13/2009**
Protocol Title: Regulation of fetal placental development in the primate	
Principal Investigator: Pepe	
Approved USDA Pain Code Level(s) – *not required for personnel additions only*: **B, C and D**	

II. PERSONNEL

List all personnel to be added to or removed from the protocol. ***All persons authorized to work on the protocol must have completed the required CITI/LATA and OHSP training modules and must have completed a health/risk assessment with the Occupational Health Department.***

Name	Added (+) or Removed (-)	E-mail Address *(additions only)*	CITI/LATA Training Certification Number *(additions only)*	CITI/LATA Training Species Module(s) Completed *(additions only)*	OHSP Training Certification Number *(additions only)*	Occupational Health/ Risk Assessment Date *(additions only)*
1.						
2.						
3.						
Procedure(s) to be performed by personnel addition #1:						
Procedure(s) to be performed by personnel addition #2:						
Procedure(s) to be performed by personnel addition #3:						

III. PROPOSED CHANGES TO THE ORIGINALLY APPROVED PROTOCOL

☐ **A. CHANGE OF SPONSOR/FUNDING SOURCE**

List the new/approved funding source, the project/grant number, the project/grant title (if different from the IACUC protocol title), and the award period.

☐ **B. CHANGE IN PROJECT SITE(S)**

List all new locations (i.e., building and room number(s)) and indicate what procedure(s) will be performed in each new location.

☐ **C. CHANGE OF SPECIES AND/OR STRAIN**

List the species to be added to or removed from the protocol. Please provide the rationale for any species/strain additions and indicate the USDA Pain Category assignment for each new species/strain. Also, list any anticipated changes in veterinary care and/or husbandry due to the new species/strain.

Species and/or strain	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E

☐ **D. CHANGE IN ANIMAL NUMBERS**

List the number of animals to be added to or removed from the protocol. If animals will be added, please provide the justification for the increase and indicate the number of animals to be assigned to each USDA Pain Category.

Species and/or strain	Year (1, 2 or 3)	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E	TOTAL

Will the animals be ordered through CompMed? _____Yes _____ No ***(Identify the source and provide the rationale/justification below.)***

Will the animals require special housing (e.g., specific bedding requirements, isolator cages, special feed, etc.)
_____ Yes ***(Please specify below.)*** _____ No

USDA Pain Code B = *Breeding or holding colony; no animal manipulation.*
USDA Pain Code C = *Procedures involving no or momentary slight pain or distress for which no pain-relieving drugs are used.*
USDA Pain Code D = *Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.*
USDA Pain Code E = *Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.*

[X] **E. CHANGE IN EXPERIMENTAL PROCEDURES AND/OR INSTRUMENTATION**

Describe, in detail, the proposed change(s). *Please indicate potentially painful/distressing effects on the animals as a result of the proposed change(s). Also, please indicate any expected adverse effects and any changes in monitoring, husbandry, housing, early endpoint, and/or experimental endpoint. Finally, please complete the checklist below.*

Currently our approved protocol allows an IVGTT test in pregnant untreated and treated animals on days 155-165 of gestation (prior to delivery). We propose to extend the gestational age to include days 80 – 165. We also request permission to perform two (2) IVGTT tests within that 65 day period to ascertain whether values for maternal IVGTT at term reflect a developmental change or are truly the result of treatment. The first test will be performed between days 80-120 of gestation, which is earlier than currently approved. The second test will be performed between days 155-165 of gestation as currently approved. There will be a minimum of 4 weeks between the first and second tests. Animals will be monitored as outlined in the approved protocol and blood sample number and volume will remain consist with the approved protocol. Both maternal IVGTTs will utilize our currently approved sampling protocol, i.e., collection of a total of 9 maternal blood samples each 3.0 ml over a 60-90 minute period, as well as a small volume (0.5 ml) drawn to flush the line and generate a "clean" sample. Moreover, total blood sampling will not exceed protocol guidelines of no more than 8 ml/kg body weight/month or 10% of total blood volume within a two week period. The mother will receive a bolus of glucose which should pose no problem to the pregnancy or the fetus. The mother will be monitored for heart rate and blood pressure, and fetal heart rate will be monitored via non-invasive ultrasound every ten minutes.

Please check all that apply to the proposed change(s). Complete the required IACUC Attachment and submit it (i.e., the original and 17 photocopies) along with the amendment form.	
Biohazardous Agents: ____ Yes ____ No (e.g., recombinant DNA, RNA, all tissue or cell samples, laboratory-induced infection, cultured pathogens)	Complete and submit **Attachment D**
Radioisotopes, *in vivo*: ____ Yes ____ No	Complete and submit **Attachment D**
Chemical Agents: ____ Yes ____ No (e.g., known or suspected chemical hazards, mutagens, or teratogens)	Complete and submit **Attachment D**
Stress or Prolonged Restraint: ____ Yes ____ No	Complete and submit **Attachment C**
Food and/or Water Deprivation: ____ Yes ____ No	Complete and submit **Attachment C**
Surgical Procedures: ____ Yes ____ No (i.e., single survival, multiple survival, or non-survival)	Complete and submit **Attachment E**
Antibody Production: ____ Yes ____ No	Complete and submit **Attachment F**
Toxicity Testing (LD50): ____ Yes ____ No	Complete and submit **Attachment G**
Lasers or Penetrating Electromagnetic Radiation: ____ Yes ____ No	
Collection of Tissues, Cells, or Organs: ____ Yes ____ No (Please explain below.)	
Tumor Transplantation or Induction: ____ Yes ____ No	

***USDA Pain Code B** = Breeding or holding colony; no animal manipulation.*
***USDA Pain Code C** = Procedures involving no or momentary slight pain or distress for which no pain-relieving drugs are used.*
***USDA Pain Code D** = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.*
***USDA Pain Code E** = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.*

Special Diet: ____ Yes ____ No (Please explain below.)	
Other : (Please specify below.)	

☐ **F. CHANGE IN ANESTHESIA, THERAPEUTICS, AND/OR EXPERIMENTAL AGENTS**

	Dose (mg/kg)	Route of Administration	Frequency of Administration	
Sedatives/Tranquilizers				
Anesthetics - General				
Anesthetics - Local				
				Length of Administration
Analgesics				
Antibiotics				
Miscellaneous				

EXPERIMENTAL AGENTS:

Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _____
_____

Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _____

***USDA Pain Code B** = Breeding or holding colony; no animal manipulation.*
***USDA Pain Code C** = Procedures involving no or momentary slight pain or distress for which no pain-relieving drugs are used.*
***USDA Pain Code D** = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.*
***USDA Pain Code E** = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.*

Agent: _____ **Agent Vehicle:** _____

Route/Site: _____ **Volume per administration:** _____

Frequency of administration: _____

Expected side effects and/or changes in animal behavior: _____

INVESTIGATOR ASSURANCES:

[x]	*I understand that it is my responsibility as the Principal Investigator to ensure that all personnel who work on this protocol are adequately trained. That training may be provided by me, an experienced member of the investigative staff, the Attending Veterinarian, a qualified Division of Comparative Medicine (CompMed) staff member, or an appropriate outside source. I hereby affirm that each person added to this protocol by way of this amendment request has been adequately trained to perform the procedure(s) indicated above. Any person who has not been adequately trained will be so trained prior to performing the procedure(s).*
[x]	*No animal involved in this project will be subjected to discomfort, pain, or distress, unless it is unavoidable in the conduct of scientifically valuable research and approval of such has been approved by the IACUC.*
[x]	*I agree to comply with all federal and institutional policies governing the use of animals used in this project.*

PRINCIPAL INVESTIGATOR:

Printed Name: Gerald J. Pepe

Signature: Gerald J. Pepe **Date:** 4/19/12

APPROVAL SIGNATURES:

PRE-VETERINARY REVIEW: *Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian before the changes are submitted to the IACUC for review.*

Attending Veterinarian Printed Name: [redacted]

Attending Veterinarian Signature: [redacted] **Date:** 4/19/12

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary slight pain or distress for which no pain-relieving drugs are used.
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USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

FINAL IACUC APPROVAL: *All revisions must be approved by the IACUC prior to implementation.* **IACUC Chair or Vice Chair Printed Name:** **IACUC Chair or Vice Chair Signature:** [redacted]	**Date:** 5/7/12

***USDA Pain Code B** = Breeding or holding colony; no animal manipulation.*
***USDA Pain Code C** = Procedures involving no or momentary slight pain or distress for which no pain-relieving drugs are used.*
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***USDA Pain Code E** = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.*

OFFICE OF RESEARCH [redacted] | Norfolk, VA 23507 | Phone: 757 [redacted] | Fax: 757 [redacted] | WWW.EVMS.EDU

EVMS
Eastern Virginia Medical School

July 1, 2010

Gerald J. Pepe, Ph.D.
Dean and Provost
Department of Physiological Sciences
Eastern Virginia Medical School
[redacted]
Norfolk, Virginia 23507

Dear Dr. Pepe: Jerry

The Institutional Animal Care and Use Committee (IACUC) has reviewed the requested information regarding your request to amend the protocol entitled, ***Regulation of Fetal-Placental Development in the Primate*** **(IACUC #09-007)**. Your original request was reviewed by the IACUC at its June 3, 2010 meeting. **The amendment (*i.e., permission to maintain sedation via cone mask inhalant rather than intubation and use of 22 mm stainless steel electrodes placed subcutaneously in the mid frontal area (vertex) and on each mastoid to ensure appropriate testing and collection of data*) has been approved via the facilitated review process.**

It is the responsibility of the Principal Investigator to notify the Committee, in writing, of any deviation from the IACUC-approved protocol. Please note that a change in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian prior to submitting the amendment request to the IACUC for approval.

It is also the responsibility of the Principal Investigator to provide for regular observation of the health of research animals and to provide the Division of Comparative Medicine (CompMed) with the name(s) and telephone number(s) of the person(s) who may be contacted and/or take action in the event that the CompMed staff notes an emergency condition with the research animals.

Thank you for your continued cooperation with the Institutional Animal Care and Use Committee.

Sincerely,

[redacted]

[redacted] *Chair*
Institutional Animal Care and Use Committee

[redacted]

cc: [redacted]
Interim Department Chair

[redacted]
Attending Veterinarian
Division of Comparative Medicine

[redacted]
Project Manager
Division of Comparative Medicine

[redacted]
Associate Dean for Research
Institutional Official

REQUEST TO AMEND A PREVIOUSLY APPROVED IACUC PROTOCOL

Institutional Animal Care and Use Committee (IACUC)

Eastern Virginia Medical School
Office of Research

IACUC Office
[redacted]

Office: 757-[redacted]
Fax: 757-[redacted]

RECEIVED
MAY 14 2010
OFFICE OF RESEARCH
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Date Received: 5/14/10 **Review Method:** X FCR / ____ FR / ____ Administrative (*Personnel Changes Only*)

IBC Approval? X Yes / ____ No **IBC Approval Date:** 4/28/10 **Final Approval Date:** ____

General Information and Instructions:

1. All requested amendments must be approved by the IACUC before they are implemented.
2. Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian before the changes are submitted to the IACUC Office.
3. A request to increase the number of animals assigned to a protocol must be approved by the IACUC before the animals are ordered.
4. Submit the original signed amendment form, along with seventeen (17) photocopies, to the IACUC Office.
5. *The IACUC reserves the right to require submission of an IACUC "Initial Review Form" based upon the Committee's assessment of the amendment(s) requested herein.*

I. ADMINISTRATIVE INFORMATION

Protocol Number:09-007	**Protocol Initial Approval Date: July 13, 2009**
Protocol Title: Regulation of Fetal-Placental Development in the Primate	
Principal Investigator: Pepe	
Approved USDA Pain Code Level(s) – *not required for personnel additions only:*	

II. PERSONNEL

List all personnel to be added to or removed from the protocol. ***All persons authorized to work on the protocol must have completed the required LATA and OHSP training modules and must have completed a health/risk assessment with the Occupational Health Department.***

Name	Added (+) or Removed (-)	E-mail Address *(additions only)*	LATA Training Certification Number *(additions only)*	LATA Training Species Module(s) Completed *(additions only)*	OHSP Training Certification Number *(additions only)*	Occupational Health/Risk Assessment Date *(additions only)*
1. [redacted]	+	[redacted]	[redacted]	Non-human primate/rabbit	[redacted]	1/22/2007 5/17/10
2. [redacted]	+	[redacted]	[redacted]	Non-human primate	[redacted]	5/2010 5/6/10
3 [redacted]	+	[redacted]	[redacted]	Non-human primate	[redacted]	4/2010 4/27/10
Procedure(s) to be performed by personnel addition #1: **Assisting / performing hearing test on baboons**						
Procedure(s) to be performed by personnel addition #2: **Assisting / performing hearing test on baboons**						
Procedure(s) to be performed by personnel addition #3: **Assisting / performing hearing test on baboons**						

III. PROPOSED CHANGES TO THE ORIGINALLY APPROVED PROTOCOL

☐ **A. CHANGE OF SPONSOR/FUNDING SOURCE**

List the new/approved funding source, the project/grant number, the project/grant title (if different from the IACUC protocol title), and the award period.

☐ **B. CHANGE IN PROJECT SITE(S)**

List all new locations (i.e., building and room number(s)) and indicate what procedure(s) will be performed in each new location.

☐ **C. CHANGE OF SPECIES AND/OR STRAIN**

List the species to be added to or removed from the protocol. Please provide the rationale for any species/strain additions and indicate the USDA Pain Category assignment for each new species/strain. Also, list any anticipated changes in veterinary care and/or husbandry due to the new species/strain.

Species and/or strain	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E

☐ **D. CHANGE IN ANIMAL NUMBERS**

List the number of animals to be added to or removed from the protocol. If animals will be added, please provide the justification for the increase and indicate the number of animals to be assigned to each USDA Pain Category.

Species and/or strain	Year (1, 2 or 3)	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E	TOTAL

Will the animals be ordered through CompMed? _____Yes _____ No ***(Identify the source and provide the rationale/justification below.)***

Will the animals require special housing (e.g., specific bedding requirements, isolator cages, special feed, etc.)
_____Yes ***(Please specify below.)*** _____ No

***USDA Pain Code B** = Breeding or holding colony; no animal manipulation.*
***USDA Pain Code C** = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.*
***USDA Pain Code D** = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.*
***USDA Pain Code E** = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.*

[X] **E. CHANGE IN EXPERIMENTAL PROCEDURES AND/OR INSTRUMENTATION**

Describe, in detail, the proposed change(s). *Please indicate potentially painful/distressing effects on the animals as a result of the proposed change(s). Also, please indicate any expected adverse effects and any changes in monitoring, husbandry, housing, early endpoint, and/or experimental endpoint. Finally, please complete the checklist below.*

I would like to have the appended research protocol (briefly outlined below) amended to my approved IACUC protocol entitled "Regulation of Fetal-Placental Development in the Primate" (#09-007). In the existing protocol, we are approved to (1) "intubate baboons with a size appropriate endotracheal tube (4-6) and to sedate animals with isoflurane" and (2) "a small patch of hair is shaved at the vertex and mastoids then a silver surface cup electrodes applied with electrode paste to the Cz and on each mastoid".

We request a change in the procedure (1) and thus maintain sedation via cone mask inhalant rather than stated intubation. We feel this is a more appropriate means of sedation for this test because the time required to complete the test is approximately 20 minutes and the procedure is essentially non-invasive. Intubation can cause throat irritation and a longer recovery time for the animal. With regard to item 2, we would like to use subcutaneous electrodes to assure appropriate testing and collection of data. Thus, we propose to use 22 mm stainless steel electrodes placed subcutaneously in the mid frontal area (vertex) and on each mastoid. All other parameters of the test will be conducted as outlined previously. Thus, BAER will be evoked and recorded using a Biologic Navigator Pro system by monaural stimulation of the ipsilateral ear at 80 dB hearing level. Alternating clicks (n= 2000) of 0.15 ms duration will be presented at a rate of 27.7/sec. Band pass filtering will be set at 100-3000Hz. In addition we will test low frequency hearing by presenting tone bursts of 80dB intensity at 500 and 1000Hz. The protocol for the tone bursts will have 1500 sweeps, a rate of 39.1/sec, a duration of 8ms, and a band pass filtering of 30-1500Hz. Once initiated, these recordings are expected to be completed over a period of 25 minutes. During the procedure, animals are kept warm (on a heat controlled heating pad and heated table) and blood pressure, heart rate, respiration, oxygen/saturation monitored as outlined in protocol #09-007. At completion of each experiment, baboons are returned to their cages and monitored to recovery stage 0 as in #09-007.

Please check all that apply to the proposed change(s). Complete the required IACUC Attachment and submit it (i.e., the original and 17 photocopies) along with the amendment form.	
Biohazardous Agents: ____ Yes ____ No (e.g., recombinant DNA, RNA, all tissue culture, laboratory-induced infection, cultured pathogens)	Complete and submit **Attachment D**
Radioisotopes, *in vivo*: ____ Yes ____ No	Complete and submit **Attachment D**
Chemical Agents: ____ Yes ____ No (e.g., known or suspected chemical hazards, mutagens, or teratogens)	Complete and submit **Attachment D**
Stress or Prolonged Restraint: ____ Yes ____ No	Complete and submit **Attachment C**
Food and/or Water Deprivation: ____ Yes ____ No	Complete and submit **Attachment C**
Surgical Procedures: ____ Yes ____ No (i.e., single survival, multiple survival, or non-survival)	Complete and submit **Attachment E**
Antibody Production: ____ Yes ____ No	Complete and submit **Attachment F**
Toxicity Testing (LD50): ____ Yes ____ No	Complete and submit **Attachment G**

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***USDA Pain Code C** = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.*
***USDA Pain Code D** = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.*
***USDA Pain Code E** = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.*

Lasers or Penetrating Electromagnetic Radiation: ____ Yes ____ No	
Collection of Tissues, Cells, or Organs: ____ Yes ____ No (Please explain below.)	
Tumor Transplantation or Induction: ____ Yes ____ No	
Special Diet: ____ Yes ____ No (Please explain below.)	
Other : (Please specify below.)	

☐ **F. CHANGE IN ANESTHESIA, THERAPEUTICS, AND/OR EXPERIMENTAL AGENTS**

	Dose (mg/kg)	Route of Administration	Frequency of Administration	Length of Administration
Sedatives/Tranquilizers				
Anesthetics - General				
Anesthetics - Local				
Analgesics				
Antibiotics				
Miscellaneous				

EXPERIMENTAL AGENTS:

Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _____

Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _____

Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _____

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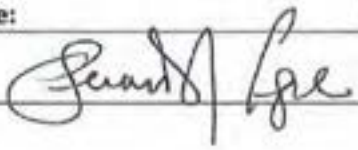
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

INVESTIGATOR ASSURANCES:	
☑	*I understand that it is my responsibility as the Principal Investigator to ensure that all personnel who work on this protocol are adequately trained. That training may be provided by me, an experienced member of the investigative staff, the Attending Veterinarian, a qualified Division of Comparative Medicine (CompMed) staff member, or an appropriate outside source. I hereby affirm that each person added to this protocol by way of this amendment request has been adequately trained to perform the procedure(s) indicated above. Any person who has not been adequately trained will be so trained prior to performing the procedure(s).*
☑	*No animal involved in this project will be subjected to discomfort, pain, or distress, unless it is unavoidable in the conduct of scientifically valuable research and approval of such has been approved by the IACUC.*
☑	*I agree to comply with all federal and institutional policies governing the use of animals used in this project.*

PRINCIPAL INVESTIGATOR:	
Printed Name:	**Gerald J. Pepe, Ph.D.**
Signature: 	**Date: 05/14/10**

APPROVAL SIGNATURES:	
PRE-VETERINARY REVIEW: *Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian before the changes are submitted to the IACUC for review.* **Attending Veterinarian Printed Name:** [redacted] **Attending Veterinarian Signature:** [redacted]	**Date:** 7/1/10
FINAL IACUC APPROVAL: *All revisions must be approved by the IACUC prior to implementation.* **IACUC Chair or Vice Chair Printed Name:** [redacted] **IACUC Chair or Vice Chair Signature:** [redacted]	**Date:** 7/6/10

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USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

OFFICE OF RESEARCH
[redacted] Norfolk, VA 23507 Phone: 757[redacted] Fax: 757[redacted]
WWW.EVMS.EDU

EVMS
Eastern Virginia Medical School

October 11, 2010

Gerald J. Pepe, Ph.D.
Dean and Provost
Department of Physiological Sciences
Eastern Virginia Medical School
[redacted]
Norfolk, Virginia 23507

Dear Dr. Pepe:

The Institutional Animal Care and Use Committee reviewed your amendment request (*i.e., permission to use a cocktail of Ketamine and Xylazine @ 7 mg/kg Ketamine and 6 mg/kg Xylazine intramuscularly to sedate Animal N026*) to the protocol entitled, ***Regulation of Fetal-Placental Development in the Primate*** **(IACUC #09-007)**, at its October 7, 2010 meeting. **The amendment to the protocol was approved.**

It is the responsibility of the Principal Investigator to notify the Committee, in writing, of any deviation from the IACUC-approved protocol. Please note that a change in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian prior to submitting the amendment request to the IACUC for approval.

It is also the responsibility of the Principal Investigator to provide for regular observation of the health of the research animals and to provide the Division of Comparative Medicine (CompMed) with the name(s) and telephone number(s) of the person(s) who may be contacted and/or take action in the event that the CompMed staff notes an emergency condition with the research animals.

Thank you for your continued cooperation with the Institutional Animal Care and Use Committee.

Sincerely,

[redacted]

[redacted] *Chair*
Institutional Animal Care and Use Committee

[redacted]

cc: [redacted]
Department Chair

[redacted]
Attending Veterinarian
Division of Comparative Medicine

[redacted]
Project Manager
Division of Comparative Medicine

[redacted]
Associate Dean for Research
Institutional Official

REQUEST TO AMEND A PREVIOUSLY APPROVED IACUC PROTOCOL

Institutional Animal Care and Use Committee (IACUC)

Eastern Virginia Medical School
Office: [redacted]
Office of Research
[redacted]

IACUC Office
[redacted]

FOR OFFICE USE ONLY

Date Received: 9/17/10 [redacted] **Review Method:** X FCR / ___ FR / ___ Administrative (*Personnel Changes Only*)

IBC Approval? X Yes / ___ No **IBC Approval Date:** 7/12/09 **Final Approval Date:** 10/21/10 [redacted]

General Information and Instructions:

1. All requested amendments must be approved by the IACUC **before** they are implemented.
2. Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian **before** the changes are submitted to the IACUC Office.
3. A request to increase the number of animals assigned to a protocol must be approved by the IACUC **before** the animals are ordered.
4. Submit the original signed amendment form, along with seventeen (17) photocopies, to the IACUC Office.
5. ***The IACUC reserves the right to require submission of an IACUC "Initial Review Form" based upon the Committee's assessment of the amendment(s) requested herein.***

I. ADMINISTRATIVE INFORMATION

Protocol Number: 09-007	**Protocol Initial Approval Date:7/13/2009**
Protocol Title: Regulation of Fetal – Placental Development in the Primate	
Principal Investigator: Pepe	
Approved USDA Pain Code Level(s) – *not required for personnel additions only*: B, C, D	

II. PERSONNEL

List all personnel to be added to or removed from the protocol. ***All persons authorized to work on the protocol must have completed the required LATA and OHSP training modules and must have completed a health/risk assessment with the Occupational Health Department.***

Name	Added (+) or Removed (-)	E-mail Address *(additions only)*	LATA Training Certification Number *(additions only)*	LATA Training Species Module(s) Completed *(additions only)*	OHSP Training Certification Number *(additions only)*	Occupational Health/ Risk Assessment Date *(additions only)*
1.						
2.						
3.						
Procedure(s) to be performed by personnel addition #1:						
Procedure(s) to be performed by personnel addition #2:						

Procedure(s) to be performed by personnel addition #3:

III. PROPOSED CHANGES TO THE ORIGINALLY APPROVED PROTOCOL

☐ **A. CHANGE OF SPONSOR/FUNDING SOURCE**

List the new/approved funding source, the project/grant number, the project/grant title (if different from the IACUC protocol title), and the award period.

☐ **B. CHANGE IN PROJECT SITE(S)**

List all new locations (i.e., building and room number(s)) and indicate what procedure(s) will be performed in each new location.

☐ **C. CHANGE OF SPECIES AND/OR STRAIN**

List the species to be added to or removed from the protocol. Please provide the rationale for any species/strain additions and indicate the USDA Pain Category assignment for each new species/strain. Also, list any anticipated changes in veterinary care and/or husbandry due to the new species/strain.

Species and/or strain	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E

☐ **D. CHANGE IN ANIMAL NUMBERS**

List the number of animals to be added to or removed from the protocol. If animals will be added, please provide the justification for the increase and indicate the number of animals to be assigned to each USDA Pain Category.

Species and/or strain	Year (1, 2 or 3)	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E	TOTAL

Will the animals be ordered through CompMed? _____Yes _____ No ***(Identify the source and provide the rationale/justification below.)***

Will the animals require special housing (e.g., specific bedding requirements, isolator cages, special feed, etc.)
_____ Yes ***(Please specify below.)*** _____ No

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

[X] **E. CHANGE IN EXPERIMENTAL PROCEDURES AND/OR INSTRUMENTATION**

Describe, in detail, the proposed change(s). *Please indicate potentially painful/distressing effects on the animals as a result of the proposed change(s). Also, please indicate any expected adverse effects and any changes in monitoring, husbandry, housing, early endpoint, and/or experimental endpoint. Finally, please complete the checklist below.*

We are requesting a change in agent used to sedate one (1) animal in particular. Animal N026 has shown signs of agitated or aggressive arousal from Ketamine sedation. The Veterinarian's been consulted and we are requesting IACUC permission to use Xylezene / Ketamine cocktail on this animal.
The dose of 6mg/kg to 7mg/kg will relieve the stress of Ketamine alone sedation.
There are no foreseen adverse effects with the use of Xylezene / Ketamine. No change in monitoring is necessary.

Please check all that apply to the proposed change(s). Complete the required IACUC Attachment and submit it (i.e., the original and 17 photocopies) along with the amendment form.	
Biohazardous Agents: ____ **Yes** ____ **No** (e.g., recombinant DNA, RNA, all tissue culture, laboratory-induced infection, cultured pathogens)	Complete and submit **Attachment D**
Radioisotopes, *in vivo*: ____ **Yes** ____ **No**	Complete and submit **Attachment D**
Chemical Agents: __ **Yes** ____ **No** (e.g., known or suspected chemical hazards, mutagens, or teratogens)	Complete and submit **Attachment D**
Stress or Prolonged Restraint: ____ **Yes** ____ **No**	Complete and submit **Attachment C**
Food and/or Water Deprivation: ____ **Yes** ____ **No**	Complete and submit **Attachment C**
Surgical Procedures: ____ **Yes** ____ **No** (i.e., single survival, multiple survival, or non-survival)	Complete and submit **Attachment E**
Antibody Production: ____ **Yes** ____ **No**	Complete and submit **Attachment F**
Toxicity Testing (LD50): ____ **Yes** ____ **No**	Complete and submit **Attachment G**
Lasers or Penetrating Electromagnetic Radiation: ____ **Yes** ____ **No**	
Collection of Tissues, Cells, or Organs: ____ **Yes** ____ **No** (Please explain below.)	
Tumor Transplantation or Induction: ____ **Yes** ____ **No**	
Special Diet: ____ **Yes** ____ **No** (Please explain below.)	

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

Other : (Please specify below.)	

[X] **F. CHANGE IN ANESTHESIA, THERAPEUTICS, AND/OR EXPERIMENTAL AGENTS**

	Dose (mg/kg)	Route of Administration	Frequency of Administration	
Sedatives/Tranquilizers				
Xylezene	6mg/kg	IM	At time of sedation for blood draw, IVGTT, ABR, Ultrasound, surgical prep, ACTH suppression study	
Ketamine	7mg/kg	IM	At time of sedation for blood draw, IVGTT, ABR, Ultrasound, surgical prep, ACTH suppression study	
Anesthetics - General				
Anesthetics - Local				
				Length of Administration
Analgesics				
Antibiotics				
Miscellaneous				

EXPERIMENTAL AGENTS:

Agent: _____ **Agent Vehicle:**

_____

Route/Site: _____ **Volume per administration:**

_____

Frequency of administration:

_____

Expected side effects and/or changes in animal behavior:

USDA Pain Code B -- Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

Agent: _____ **Agent Vehicle:** _____

Route/Site: _____ **Volume per administration:** _____

Frequency of administration: _____

Expected side effects and/or changes in animal behavior: _____

Agent: _____ **Agent Vehicle:** _____

Route/Site: _____ **Volume per administration:** _____

Frequency of administration: _____

Expected side effects and/or changes in animal behavior: _____

INVESTIGATOR ASSURANCES:

X	*I understand that it is my responsibility as the Principal Investigator to ensure that all personnel who work on this protocol are adequately trained. That training may be provided by me, an experienced member of the investigative staff, the Attending Veterinarian, a qualified Division of Comparative Medicine (CompMed) staff member, or an appropriate outside source. I hereby affirm that each person added to this protocol by way of this amendment request has been adequately trained to perform the procedure(s) indicated above. Any person who has not been adequately trained will be so trained prior to performing the procedure(s).*
X	*No animal involved in this project will be subjected to discomfort, pain, or distress, unless it is unavoidable in the conduct of scientifically valuable research and approval of such has been approved by the IACUC.*
X	*I agree to comply with all federal and institutional policies governing the use of animals used in this project.*

PRINCIPAL INVESTIGATOR:

Printed Name:

Signature: _____ **Date:** 9/14/10

USDA Pain Code B – Breeding or holding colony; no animal manipulation.
USDA Pain Code C – Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D – Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E – Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

APPROVAL SIGNATURES:	
PRE-VETERINARY REVIEW: *Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian before the changes are submitted to the IACUC for review.* **Attending Veterinarian Printed Name:** [redacted] **Attending Veterinarian Signature:** [redacted]	**Date:** 9/14/10
FINAL IACUC APPROVAL: *All revisions must be approved by the IACUC prior to implementation.* **IACUC Chair or Vice Chair Printed Name:** [redacted] **IACUC Chair or Vice Chair Signature:** [redacted]	**Date:** 10/14/10

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

EVMS
Eastern Virginia Medical School

August 11, 2014

Gerald J. Pepe, Ph.D.
Chair, Department of Physiological Sciences
Eastern Virginia Medical School
[redacted]
Norfolk. Virginia 23507

Dear Dr. Pepe:

The Institutional Animal Care and Use Committee reviewed your amendment request (*i.e., permission to collect skeletal muscle samples using a scalpel blade instead of punch biopsy; the analgesic agent, Banamine®, will be administered post-operatively*) to the protocol entitled, ***Estrogen Regulation of Insulin Secretion and Signaling in the Non-pregnant Baboon*** **(IACUC #12-007)**, at its August 7, 2014 meeting. **The amendment to the protocol was approved.**

Although the Division of Comparative Medicine (CompMed) will make every effort to provide housing, husbandry, and veterinary support for your research project, IACUC approval of this amendment request does not guarantee that CompMed will be able to accommodate the work immediately. Available space and resources within the CompMed animal facility are determined by the number of ongoing projects and the animal census at any given time. Please consult with the CompMed management team to confirm availability prior to implementing the approved modification(s).

It is the responsibility of the Principal Investigator to notify the Committee, in writing, of any deviation from the IACUC-approved protocol. Please note that a change in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian prior to submitting the amendment request to the IACUC for approval.

It is also the responsibility of the Principal Investigator to provide for regular observation of the health of the research animals and to provide the Division of Comparative Medicine (CompMed) with the name(s) and telephone number(s) of the person(s) who may be contacted and/or take action in the event that the CompMed staff notes an emergency condition with the research animals.

Thank you for your continued cooperation with the Institutional Animal Care and Use Committee.

Sincerely,

[redacted]
[redacted] *Chair*
Institutional Animal Care and Use Committee

Office of Research
[redacted]
NORFOLK, VA 23507
tel 757 [redacted]
fax 757 [redacted]
www.evms.edu

[redacted]

cc: [redacted]
Attending Veterinarian
Division of Comparative Medicine

[redacted]
Project Manager
Division of Comparative Medicine

[redacted]
Senior Associate Dean for Research
Institutional Official

PHYSIOLOGICAL SCIENCES
P.O. Box 1980 Norfolk, VA 23501-1980 | Phone- 757 [redacted] | Fax: [redacted]
WWW.EVMS.EDU

EVMS
Eastern Virginia Medical School

Gerald J. Pepe, PhD
Professor and Chair

July 15, 2014

JUL 23 2014

[redacted]
Institutional Animal Care and Use Committee
Eastern Virginia Medical School
Norfolk, VA 23507

Dear [redacted]

I would like to have the appended research protocols (briefly outlined below) amended to my approved IACUC protocols entitled "Regulation of Fetal-Placental Development in the Primate" (#12-010) and Estrogen Regulation of Insulin Signaling in the Non-Pregnant Baboon" (#12-007). *Amendment 1*: In both #12-010 and #12-007 IACUC protocols, we are approved to obtain biopsies of skeletal muscle from adolescent and adult baboons using a punch biopsy. We have found that this procedure yields non-uniform threads of muscle fibers which are almost impossible to embed in paraffin. As such we cannot perform requisite histologic and immuno-histochemical analyses thereby limiting conduct of the approved studies. Accordingly, we are requesting permission to collect muscle biopsies using a small scalpel. This would allow us to obtain a uniform sample which we can orient appropriately in paraffin to permit histology studies as well as provide sufficient tissue that can be frozen (liquid nitrogen) for subsequent biochemical analyses (e.g. Western blot; RT-PCR). *Amendment 2*: In both protocols #12-010 and #12-007, we are approved to perform intravenous glucose tolerance tests (iv GTT) but only in protocol #12-007 are we approved to obtain muscles biopsies before (time 0) and at 30 minutes after onset of iv glucose infusion. As insulin levels rise rapidly after onset of glucose infusion, the second biopsy permits analysis of response of muscle to endogenous insulin i.e. compare expression of proteins in the 0 time and 30 minute samples. Preliminary studies (previous amendment approved July, 2012) confirmed these time points are appropriate for the studies proposed. Accordingly, I am requesting that we conduct this multiple biopsy procedure during conduct of two iv GTT experiments performed in adolescent baboons first at 3-4 years of age and subsequently at 6-10 years of age.

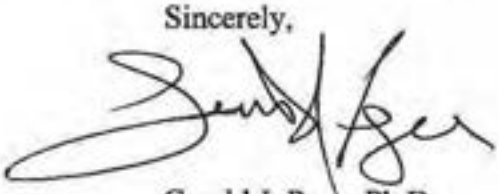
To summarize the muscle biopsy procedure, the area of skin overlying the vastus lateralis muscle is shaved and prepared using sterile procedures as outlined previously. A small incision is made through the skin with a #10 surgical blade to access the underlying muscle for biopsy. We are requesting that this incision be increased from 0.5 cm to 1 cm. A 2.5-3.0 cm (length) x 0.50- 0.75 cm (width) by 0.20-0.25 cm (depth) segment of skeletal muscle will then be surgically isolated using a #10 surgical blade. Hemostasis will be maintained with direct pressure and the muscle area packed with gel foam and the superficial incision closed with absorbable suture. For experiments in which 0 and 30 minute samples are collected, the first will be obtained from the

right (or left) limb and the second from the alternate limb. Finally, we will continue to follow the post-operative recovery/analgesia protocols as currently approved in #12-010 and #12-007.

I have also appended a summary manuscript (Patel et al, 2011; open access journal) and abstracts of experiments (Edgett et al, 2013; Cobley et al, 2014) in which comparable procedures have been used to obtain multiple muscle biopsies in human studies; biopsies up to 290 mg have been collected. Also, please note Figure 5 (Patel et al, 2011) which shows isolation of a sample of vastus lateralis that appears (on computer view) to exceed the size (dimensions) we are requesting in the current study. That manuscript also describes (Table 1) the level of pain (VAS pain score 0 = no pain; 100 = as bad as it can be) perceived during (median 7; range 1-34) and 1 and 7 days after the procedure (median 4 and 1, respectively). Complications were minimal and when reported were often wound hematomas.

The IACUC's time and effort in reviewing these amendments is most appreciated.

Sincerely,



Gerald J. Pepe, Ph.D.

REQUEST TO AMEND A PREVIOUSLY APPROVED IACUC PROTOCOL

Institutional Animal Care and Use Committee (IACUC) JUL 23 2014

Eastern Virginia Medical School
Office of Research
IACUC Office
[redacted]
Office: 757-[redacted]
Fax: 757-[redacted]

FOR OFFICE USE ONLY [redacted]
Date Received: 7/23/14 **Review Method:** X FCR / ____ FR / ____ Administrative (*Personnel Changes Only*)
IBC Approval? ____ Yes / X No **IBC Approval Date:** N/A **Final Approval Date:** 8/2/14 [redacted]

General Information and Instructions:

1. All requested amendments must be approved by the IACUC before they are implemented.
2. Changes in administration, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian before the changes are submitted to the IACUC Office.
3. A request to increase the number of animals assigned to a protocol must be approved by the IACUC before the animals are ordered.
4. Submit the original signed amendment form to the IACUC Office located in [redacted] and e-mail the MSWord version of the form to the IACUC Administrator.
5. *The IACUC reserves the right to require submission of an IACUC "Initial Review Form" based upon the Committee's assessment of the amendment(s) requested herein.*

I. ADMINISTRATIVE INFORMATION

Protocol Number: 12-007	**Protocol Initial Approval Date: 7-16-2012**
Protocol Title: Estrogen regulation of insulin secretion and signaling in the non-pregnant baboon	
Principal Investigator: Gerald J Pepe, PHD	
Approved USDA Pain Code Level(s) – *not required for personnel additions only*: **D**	

II. PERSONNEL

List all personnel to be added to or removed from the protocol. All persons authorized to work on the protocol must have completed the required CITI/LATA and OHSP training modules and must have completed a health/risk assessment with the Occupational Health Department.

Name	Added (+) or Removed (-)	E-mail Address *(additions only)*	CITI/LATA Training Certification Number *(additions only)*	CITI/LATA Training Species Module(s) Completed *(additions only)*	OHSP Training Certification Number *(additions only)*	Occupational Health/ Risk Assessment Date *(additions only)*
1.						
2.						
3.						
Procedure(s) to be performed by personnel addition #1:						
Procedure(s) to be performed by personnel addition #2:						
Procedure(s) to be performed by personnel addition #3:						

III. PROPOSED CHANGES TO THE ORIGINALLY APPROVED PROTOCOL

☐ **A. CHANGE OF SPONSOR/FUNDING SOURCE**

List the new/approved funding source, the project/grant number, the project/grant title (if different from the IACUC protocol title), and the award period.

☐ **B. CHANGE IN PROJECT SITE(S)**

List all new locations (i.e., building and room number(s)) and indicate what procedure(s) will be performed in each new location.

☐ **C. CHANGE OF SPECIES AND/OR STRAIN**

List the species to be added to or removed from the protocol. Please provide the rationale for any species/strain additions and indicate the USDA Pain Category assignment for each new species/strain. Also, list any anticipated changes in veterinary care and/or husbandry due to the new species/strain.

Species and/or strain	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E

☐ **D. CHANGE IN ANIMAL NUMBERS**

List the number of animals to be added to or removed from the protocol. If animals will be added, please provide the justification for the increase and indicate the number of animals to be assigned to each USDA Pain Category.

Species and/or strain	Year (1, 2 or 3)	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E	TOTAL

Will the animals be ordered through CompMed? _____Yes _____ No ***(Identify the source and provide the rationale/justification below.)***

Will the animals require special housing (e.g., specific bedding requirements, isolator cages, special feed, etc.)
_____ Yes ***(Please specify below.)*** _____ No

***USDA Pain Code B** = Breeding or holding colony; no animal manipulation.*
***USDA Pain Code C** = Procedures involving no or momentary slight pain or distress for which no pain-relieving drugs are used.*
***USDA Pain Code D** = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.*
***USDA Pain Code E** = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.*

[X] **E. CHANGE IN EXPERIMENTAL PROCEDURES AND/OR INSTRUMENTATION**

Describe, in detail, the proposed change(s). *Please indicate potentially painful/distressing effects on the animals as a result of the proposed change(s). Also, please indicate any expected adverse effects and any changes in monitoring, husbandry, housing, early endpoint, and/or experimental endpoint. Finally, please complete the checklist below.*

We are currently approved to obtain muscle biopsy during IVGT test. We request the following change in procedure.

Amendment 1: We would like to obtain skeletal muscle samples using a scalpel blade rather than the currently approved punch biopsy. The skin area is shaved and sterilized as currently approved. A small incision (0.5cm – 1cm) is made exposing the vastus lateralis. At time '0' and '30', a 2.5-3.0cm(L) x 0.5-0.75cm(W) x 0.2-0.25(D) segment of muscle is surgically removed from alternating legs. The area is packed with gel foam to minimize bleeding. The skin is then closed with absorbable suture.

Post-op analgesia (Banamine) would be given at completion of the glucose test and then 2days BID as currently approved. Animals will be monitored for appetite, fecal output and overall change in behavior. The veterinarian will be contacted if pain medication is needed beyond the 2days post procedure.

Please check all that apply to the proposed change(s). Complete the required IACUC Attachment and submit it along with the amendment form.	
Biohazardous Agents: ____ Yes __X__ No (e.g., recombinant DNA, RNA, all tissue or cell samples, laboratory-induced infection, cultured pathogens)	Complete and submit **Attachment D**
Radioisotopes, *in vivo*: ____ Yes __X__ No	Complete and submit **Attachment D**
Chemical Agents: ____ Yes _X___ No (e.g., known or suspected chemical hazards, mutagens, or teratogens)	Complete and submit **Attachment D**
Stress or Prolonged Restraint: ____ Yes __X_ No	Complete and submit **Attachment C**
Food and/or Water Deprivation: ____ Yes _X___ No	Complete and submit **Attachment C**
Surgical Procedures: ____ Yes _X___ No (i.e., single survival, multiple survival, or non-survival)	Complete and submit **Attachment E**
Antibody Production: ____ Yes __X__ No	Complete and submit **Attachment F**
Toxicity Testing (LD50): ____ Yes __X__ No	Complete and submit **Attachment G**
Lasers or Penetrating Electromagnetic Radiation: ____ Yes __X__ No	
Collection of Tissues, Cells, or Organs: _X___ Yes ____ No (Please explain below.) Skeletal Muscle will be obtained during the experiment	

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USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

Tumor Transplantation or Induction: ____ Yes __X__ No	
Special Diet: ____ Yes __X_ No (Please explain below.)	
Other : N/A (Please specify below.)	

☐ F. CHANGE IN ANESTHESIA, THERAPEUTICS, AND/OR EXPERIMENTAL AGENTS

	Dose (mg/kg)	Route of Administration	Frequency of Administration	
Sedatives/Tranquilizers				
Anesthetics - General				
Anesthetics - Local				
				Length of Administration
Analgesics				
Flunixin meglumine (Banamine)	**2mg / kg**	**IM**	**Completion of glucose test and 2days BID**	**3days total (PRN with consult from vet)**
Antibiotics				
Miscellaneous				

EXPERIMENTAL AGENTS:

Agent: _____ **Agent Vehicle:** _____

Route/Site: _____ Volume per administration: _____

Frequency of administration: _____

Expected side effects and/or changes in animal behavior: _____

_____

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary slight pain or distress for which no pain-relieving drugs are used.
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USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

INVESTIGATOR ASSURANCES:	
X	*I understand that it is my responsibility as the Principal Investigator to ensure that all personnel who work on this protocol are adequately trained. That training may be provided by me, an experienced member of the investigative staff, the Attending Veterinarian, a qualified Division of Comparative Medicine (CompMed) staff member, or an appropriate outside source. I hereby affirm that each person added to this protocol by way of this amendment request has been adequately trained to perform the procedure(s) indicated above. Any person who has not been adequately trained will be so trained prior to performing the procedure(s).*
X	*No animal involved in this project will be subjected to discomfort, pain, or distress, unless it is unavoidable in the conduct of scientifically valuable research and approval of such has been approved by the IACUC.*
X	*I agree to comply with all federal and institutional policies governing the use of animals used in this project.*

PRINCIPAL INVESTIGATOR:	
Printed Name: Gerald J. Pepe, PH.D.	
Signature: Gerald J Pepe	**Date:** 7/15/2014

APPROVAL SIGNATURES:	
VETERINARY PRE-REVIEW: *Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian before the changes are submitted to the IACUC for review.* **Attending Veterinarian Printed Name:** [redacted] **Attending Veterinarian Signature:** [redacted]	**Date:**
FINAL IACUC APPROVAL: *All revisions must be approved by the IACUC prior to implementation.* **IACUC Chair or Vice Chair Printed Name:** [redacted] **IACUC Chair or Vice Chair Signature:** [redacted]	8-12-2014 **Date:**

USDA Pain Code B = Breeding or holding colony; no anim
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USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

Attending Veterinarian Printed Name: [redacted] **Attending Veterinarian Signature:**	**Date:**
FINAL IACUC APPROVAL: *All revisions must be approved by the IACUC prior to implementation.* **IACUC Chair or Vice Chair Printed Name:** **IACUC Chair or Vice Chair Signature:**	**Date:**

***USDA Pain Code B** = Breeding or holding colony; no animal manipulation.*
***USDA Pain Code C** – Procedures involving no or momentary slight pain or distress for which no pain-relieving drugs are used.*
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***USDA Pain Code E** = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.*

OFFICE OF RESEARCH | WWW.EVMS.EDU
[redacted] Norfolk, VA 23507 | Phone: [redacted] Fax [redacted]

EVMS
Eastern Virginia Medical School

July 16, 2012

Gerald J. Pepe, Ph.D.
Chair, Department of Physiological Sciences
Eastern Virginia Medical School
[redacted]
Norfolk, Virginia 23507

Dear Dr. Pepe:

Your response to the April 11, 2012 letter regarding the protocol entitled, ***Estrogen Regulation of Insulin Secretion and Signaling in the Non-pregnant Baboon*** **(IACUC #12-007)**, has been reviewed and accepted by the Institutional Animal Care and Use Committee. **The project is now approved for one year.** Continued approval beyond this point will require submission of an annual progress report, no later than **June 10, 2013**.

The following requests sent to the IACUC via your correspondence dated July 5, 2012 have also been approved:

1. **permission to transfer one of four animals assigned to another protocol and slated to be euthanized because it has reached the multi-survival surgery limit or has other issues that prevent it from use in the parent protocol to 1) perform the necessary procedure to assess the likelihood of excessive blood loss and the potential for inducing pancreatitis as a result of performing the procedures approved in this protocol, and 2) obtain a skeletal muscle biopsy.**
2. **permission to allow the animal to recover from the biopsy procedure and to be sustained for 24-36 hours post-recovery to monitor for uncontrolled bleeding. It is the understanding of the Committee that the animal will be euthanized at the end of the 24-36 hours observation period.**

Although the Division of Comparative Medicine (CompMed) will make every effort to provide housing, husbandry, and veterinary support for your research project, IACUC approval of this protocol does not guarantee that CompMed will be able to accommodate the work immediately. Available space and resources within the CompMed animal facility are determined by the number of ongoing projects and the animal census at any given time. Please consult with the CompMed management team to confirm the project start date.

It is the responsibility of the principal investigator to notify the Committee, in writing, of any deviation from the IACUC-approved protocol. Please note that a change in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian prior to submitting the amendment request to the IACUC for approval.

It is also the responsibility of the principal investigator to provide for regular observation of the health of the research animals and to provide CompMed with the name(s) and telephone number(s) of the person(s) who may be contacted and/or take action in the event that the CompMed staff notes an emergency condition with the research animals.

Thank you for your continued cooperation with the Institutional Animal Care and Use Committee.

Sincerely,

[redacted]

[redacted], *Chair*
Institutional Animal Care and Use Committee

[redacted]

cc: [redacted]
Attending Veterinarian
Division of Comparative Medicine

[redacted]
Program Manager
Division of Comparative Medicine

[redacted]
Associate Dean for Research
Institutional Official

EASTERN VIRGINIA MEDICAL SCHOOL
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

FOR IACUC USE ONLY:	
IACUC Number: 12-007	Review Date(s):
NOTES: Revision #1 / FINAL	Final Approval Date: 7/16/12
	Progress Report Due:

Submission Instructions: Submit the original signed typed form to the IACUC Office located in [redacted] and e-mail the MSWord version of the form to the IACUC Administrator no later than 5:00 p.m. on the submission deadline date. **Forms received after the submission deadline will be held for review at the next IACUC meeting.** For assistance, please contact the IACUC Administrator at [redacted].

Initial Review Form for New Animal Care and Use Protocols

PROJECT TITLE: *(If the project title is different from the grant title, please list both titles below.)*
Estrogen regulation of insulin secretion and signaling in the non-pregnant baboon

Is this a 3-year renewal of an existing IACUC protocol?	☒ NO	☐ YES	Related IACUC #	

SPECIES INFORMATION: (***In addition to the species, please list the strain(s), if applicable, the sex(es,) and the age(s) of the animals.***) **Adult female (7-15 years old) and male (8-20 years old) baboons as well as offspring born to animals in the colony and studied in the pre-and post pubertal period and as adults**
Baboon (papio anubis/cynocephalus) females. age 7-12 years old

Principal Investigator:	Gerald J. Pepe, Ph.D.		
Mailing Address:	Department of Physiological Sciences		
	[redacted] Norfolk, VA 23507		
Phone: Office	[redacted]	Home:	[redacted]
Lab:	[redacted]	E-mail:	[redacted]@evms.edu

Animal Emergency Contact Person:	[redacted]		
Phone: Office:	[redacted]	Home:	[redacted]
Lab:	[redacted]	E-mail:	[redacted]@evms.edu

Technical Coordinator:			
Phone: Office:		Home:	
Lab:		E-mail:	

Co-Investigator #1:			
Phone: Office		Home:	
Lab:		E-mail:	

Co-Investigator #2:			
Phone: Office:		Home:	
Lab:		E-mail:	

Co-Investigator #3:			
Phone: Office:		Home:	
Lab:		E-mail:	

Co-Investigator #4:			
Phone: Office:		Home:	
Lab:		E-mail:	

LIST ALL PROJECT SITES:				LIST THE PROJECT PERIOD:	
Bldg:	[redacted]	Room(s):	[redacted]	From: 8/1/12	To: 7/31/15
Bldg:	[redacted]	Room(s):	[redacted]		

FUNDING SOURCE(S):	***Please check all that apply.*** ☐ Federal Government ☐ State or Other Government ***Specify Source:*** _____ ☐ Private ☐ Industry ☒ Campus/Department Funds ☒ Other ***Specify source:*** Start up		
STATUS OF FUNDING:	☐ Approved	☐ Pending	☐ Not Applicable
Is a committee approval verification letter needed for the funding source(s)?	☒ NO Please note that it is the investigator's responsibility to inform the funding agency of any changes to the animal protocol. Any changes must also be approved by the IACUC before they are implemented.	☐ YES (***Complete Attachment A, REQUEST FOR A LETTER OF VERIFICATION***) ☐ Final copy of grant attached Please include a final copy of the grant to permit comparison of the animal work described in the grant with the animal work described in the *Initial Review Form*.	

OTHER COMMITTEE REVIEWS:

Prior to initiation of this project, approval must be acquired from the appropriate committees or offices.

Please complete the following table as it pertains to your protocol. If applicable, complete ***Attachment D, USE OF HAZARDOUS AGENTS***.

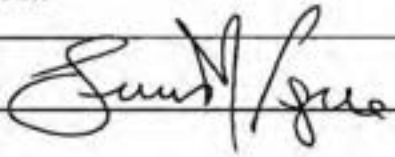
Project Involves:	Yes	No	Committee/Office	Certification Number	Hazard to:	
					Personnel	Animals
Radioisotopes, *in vivo*		X	EVMS Radiation Safety Committee *(Complete **Attachment D**)*			
Recombinant DNA, RNA, All Tissue or Cell Samples, Laboratory-Induced Infection, or Cultured Pathogens		X	EVMS Institutional Biosafety Committee *(Complete **Attachment D**)*			
Known or Suspected Chemical Hazards, Mutagens or Teratogens	X		EVMS Office of Environmental Health & Safety/Radiation Safety *(Complete **Attachment D**)*			
Lasers or Penetrating Electromagnetic Radiation with Living Animals		X	EVMS Office of Environmental Health & Safety/Radiation Safety			
Other (*Please describe*):		X				

PRINCIPAL INVESTIGATOR'S ASSURANCES:

I hereby certify that:

- no animal involved in this project will be subjected to discomfort, pain, or distress, unless it is unavoidable in the conduct of scientifically valuable research;
- any such discomfort, pain, or distress will be alleviated with the appropriate anesthetic, analgesic, or tranquilizing drugs, unless specific approval for not using these agents is given by the Committee;
- the project will be carried out within the provisions of the Animal Welfare Act (Public Law 99-198), the National Research Council (NRC), the Public Health Service Policy on Humane Care and Use of Laboratory Animals (PHS), the "Guide for the Care and Use of Laboratory Animals (1996)", the Health Research Extension Act of 1985 Public Law 99-158 (11/20/86), and United States Department of Agriculture (USDA) regulations;
- all procedural and/or personnel changes will be brought to the attention of the IACUC through the amendment process, prior to implementation, understanding that failure to request an amendment for changes in animal use may place me and the Institution in violation of federal regulations and the Animal Welfare Act;
- the details of the research to be conducted in this protocol are consistent with the details of the research as written in any grant, contract, or subcontract related to or connected with this protocol;
- all personnel using animals have completed the appropriate training requirements to assure the humane, safe, and appropriate use of animals in this context.

The signatures below signify assurance that the individuals involved will comply with the project as described herein.

Principal Investigator:		Date:	July 3, 2012
Technical Coordinator:		Date:	
Co-Investigator #1:		Date:	
Co-Investigator #2:		Date:	
Co-Investigator #3:		Date:	
Co-Investigator #4:		Date:	

DEPARTMENT CHAIR'S ENDORSEMENT:
I have reviewed the research project which is the basis for this submission. I am aware of and approve of the procedures outlined for this study. I agree to take ultimate fiscal responsibility for the payment of animal care charges.

Department Chair or Designee:	[redacted]	Date:	7/5/12
Printed or Typed Name:	[redacted]		

VETERINARY CONSULTATION:
The IACUC requires a **MANDATORY** consultation with the veterinarian to provide the investigator with information that is relevant to the species and study procedures. It is the responsibility of the investigator to incorporate the appropriate information into the protocol **BEFORE** it is submitted to the IACUC for review. The veterinarian's signature below **does not** constitute an approval of the protocol. The signature merely acknowledges that a consultation with the veterinarian has occurred.

EVMS Veterinarian		Date:	
Printed or Typed Name:	[redacted]		

IACUC APPROVAL:

IACUC Chair or Designee:	[redacted]	Date:	
Printed or Typed Name:	[redacted]		7/20/12

DEPARTMENT CHAIR'S ENDORSEMENT:
I have reviewed the research project which is the basis for this submission. I am aware of and approve of the procedures outlined for this study. I agree to take ultimate fiscal responsibility for the payment of animal care charges.

Department Chair or Designee:		Date:	3/19/12
Printed or Typed Name:	Gerald J. Pepe, Ph.D.		

VETERINARY CONSULTATION:
The IACUC requires a **MANDATORY** consultation with the veterinarian to provide the investigator with information that is relevant to the species and study procedures. It is the responsibility of the investigator to incorporate the appropriate information into the protocol **BEFORE** it is submitted to the IACUC for review. The veterinarian's signature below **does not** constitute an approval of the protocol. The signature merely acknowledges that a consultation with the veterinarian has occurred.

EVMS Veterinarian	[redacted]	Date:	3/8/12
Printed or Typed Name:			

IACUC APPROVAL:

IACUC Chair or Designee:		Date:	
Printed or Typed Name:			

A. PROTOCOL OBJECTIVE:

In clear, concise, non-technical, lay language *(i.e. the type of writing style used in newspapers)*, summarize the background, general hypothesis, experimental plan, and relevance of the study to the advancement of scientific knowledge and/or the benefits to human and animal health. All abbreviations must be defined. **Scientific abstracts from grant applications or journal articles are not acceptable.**

The incidence of diabetes and associated cardiovascular disease in humans is increasing at an alarming rate in the United States. Diabetes is exhibited as Type 1 in which the pancreas loses the ability to produce insulin or type 2 in which the tissues of the body are insensitive to the action of insulin. As a consequence carbohydrate, i.e. glucose, is not taken up and used by the cells resulting in elevated levels of glucose, i.e. hyperglycemia in the blood stream, which in turn leads to complications in cardiovascular and kidney function, as well as increased incidence of inflammation and infection. Despite the epidemic level of diabetes, treatment options are often difficult to administer and the mechanisms which mediate the production of insulin by the pancreas and action of insulin on target cells are incompletely understood. Improving our understanding of insulin secretion and action is expected to lead to new therapies for reducing the incidence and improving the treatment of diabetes. A gender-specific difference in the incidence of diabetes exists between women and men, in which premenopausal women exhibit a lower incidence of diabetes than men, but this benefit disappears in women after menopause. Moreover, estrogen which is produced prior to menopause decreases the incidence of diabetes in post-menopausal women and promotes insulin action/sensitivity and glucose tolerance in laboratory rodents. However, very little is known about the mechanisms which underlie estrogen action on insulin secretion/action. Our laboratory has shown that the baboon provides an excellent translational model, particularly regarding the role of estrogen on essential physiological processes. The present study is designed to establish the effect of and mechanisms underlying the action of estrogen on insulin secretion and action/signaling in the female baboon. To accomplish this objective, baboons will be studied during the normal menstrual cycle when estrogen levels are elevated and after removal of the ovaries, which produce estrogen, and supplementation with estrogen for 14 days to replicate levels of the normal cycle or acutely to assess rapid actions of this steroid hormone. During each treatment protocol a glucose tolerance test will be performed to assess the capacity for insulin secretion and action and biopsies of the pancreas, fat (i.e. adipose) and muscle tissue will be taken to determine the molecular mechanisms/steps which insulin takes to maintain normal levels of glucose in the blood. Results from this study in the baboon are expected to translate to the human and improve our understanding of how estrogen acts to regulate insulin secretion/action to maintain normal glucose levels.

B. SEARCH FOR ALTERNATIVES:

In an effort to minimize pain and distress, the Animal Welfare Act (AWA) regulations require principal investigators to consider alternatives to procedures that may cause more than momentary or slight pain or distress to animals. The AWA also requires principal investigators to provide a written narrative of the methods used and sources consulted to determine the availability of alternatives, including replacements, reductions, and refinements of animal use. These alternatives should be consistent with the goals of the proposed research. *Potential alternatives that do not allow the attainment of the goals of the proposed research are not, by definition, alternatives.* The "3 Rs" are defined below:

REPLACEMENT: *An alternative that will be equally informative. Replacements include, but are not limited to, in vitro models, in silico methods, invertebrate models, and vertebrate models.*

REDUCTION: *Reducing the number of animals to the minimum required to obtain scientifically valid data and demonstrating that the proposed research does not unnecessarily duplicate previous work.* Reduction includes statistical methods to reduce animal numbers, and it addresses whether or not animals can be reused for other purposes.

REFINEMENT: *A procedure that lessens or eliminates pain or distress, thereby enhancing animal well-being.* Housing, environmental enrichment, animal identification, anesthesia, analgesia, and euthanasia procedures can be refined, in addition to activities normally thought of as procedures, such as surgeries, tissue or fluid collection, etc.

The fundamental goal of the AWA and USDA Policy 12 (June 21, 2000) is to minimize pain and distress to animals; consequently, the regulations state that any proposed animal activity or significant changes to an ongoing animal activity must include the following: (1) a rationale for involving animals, and the appropriateness of the species and the number of animals to be used; (2) a description of the procedures or methods designed to assure that discomfort and pain to animals will be limited to that which is unavoidable in the conduct of scientifically valuable research, and that analgesic, anesthetic, and tranquilizing drugs will be used where indicated and appropriate to minimize discomfort and pain to animals; (3) a written narrative description of the methods and sources used to consider alternatives to procedures that may cause more than momentary or slight pain or distress to the animals, and; (4) the written assurance that the activities do not unnecessarily duplicate previous experiments.

DATABASE SEARCHES

A database search is considered to be the most effective and efficient method for demonstrating compliance with the federal regulations for consideration of alternatives to painful and distressful procedures, although other sources, such as conferences, colloquia, subject expert consultation, etc., may provide relevant and up-to-date information regarding alternatives, in lieu of or in addition to a database search. **Institutional policy requires investigators to specify at least two (2) databases or other acceptable sources** that were used to determine that alternatives to animals have been considered, that the minimal number of animals have been requested, that the proposed research is not duplicative of previous work, and that alternatives to procedures that may cause more than momentary or slight pain or distress to the animals have been considered. For all database searches, the following information **must** be provided. (1) the name of the database; (2) the date the search was performed; (3) the time period covered by the search, and; (4) the key words and/or the search strategy used.

Please be sure to list all key words and key word combinations used and the number of citations found for each key word or combination [*e.g. amiloride mouse kidney (455 citations), mouse hemizona assay (453 citations)*]. **PLEASE NOTE: The search must include the key word "pain" and any relevant combination thereof.** Be sure to search for all applicable terms, including the search for alternatives [*e.g. mouse heart computer model (55 citations)*]. Use the widest possible time range to include both modern and classical references.

EXPERT CONSULTATIONS

An appropriate, well documented consultation with an expert in the field of the proposed research can replace a second database search. In order to demonstrate to the IACUC the expert's knowledge of the availability of alternatives in the specific field of study, documentation of the consultation **must** include the following: (1) the consultant's name and qualifications, and (2) the date and content of the consultation.

DESCRIPTION AND JUSTIFICATION

Regardless of the sources used to search for alternatives, the written narrative should include adequate information for the IACUC to assess that a reasonable and good faith effort was made to determine the availability of alternatives to animals or procedures. If the database search or another acceptable source identifies an alternative that could be used to accomplish the goals of the proposed research, however, the investigator chooses not to use that alternative, the investigator must provide a written narrative justifying why the alternative was not used.

1. **Database and Literature Searches:**

	Yes (X)	Date Search Conducted	Key Words/Search Strategy	Time Period Covered by Search
Databases/Computer Systems				
AGRICOLA Database (National Agriculture Libr.)	x	3/16/12	Estrogens (9254); insulin secretion/diabetes , etc (45871);menstrual cycle (1014); ovariectomy (2675);laparoscopy (979); laparotomy (575); biopsy (7405); skeletal muscle tissue (9000); adipose tissue (16476); pancreatic tissue (5191); papio/baboon (755); non-human primates (1402); animal use alternatives, animal welfare, animal models, pain/ stress/distress/suffering, refine/replace/ reduce, humane endpoint, etc (166637). Citations =1,0	1970-2012
MEDLINE Database	x	3/16/12	Estrogens (180116); insulin secretion/ diabetes , etc (542016);menstrual cycle (41305); ovariectomy (32576); laparoscopy (77373); laparotomy (42033); biopsy (365119); skeletal muscle tissue (236203); adipose tissue (91066); pancreatic tissue (410610); papio/baboon (12789); non-human primates (16614); animal use alternatives, animal welfare, animal models, anesthesia/ analgesia, pain/stress/distress/ suffering, refine/replace/reduce, humane endpoint, etc (7702069). Citations =10,0	1946-2012
CAB Abstracts Database				
TOXLINE Database				
BIOSIS Database				
Other: EMBASE				
Literature and Reference Sources				
AAALAS				
Quick Biblio. Series (AGRICOLA)				

Laboratory Animal Welfare Biblio (NLM)				
Animal Welfare Information Ctr.				

2. List any consultations with investigators in the field. (*This consult should be related to replacements, reductions, and/or refinements and not simply to the science behind the research.*)

Dr. Pepe has met on several occasions with [redacted], Chair of Internal Medicine here at EVMS and an acknowledged expert in Diabetes and potential underlying causes. The animal protocol described in this proposal was in fact developed in consultation with [redacted]. In addition, Dr. Pepe recently met with other experts here at EVMS in diabetes to ask their opinion and analysis/critique of the experimental goals and animal protocol. This meeting included [redacted] (Assistant Professor, Internal Medicine; Diabetes/beta cell expertise) and [redacted] (Associate Professor, Physiological Sciences; expertise in fat/obesity/diabetes).

3. Provide a brief narrative regarding search methods utilized, but not listed above.

C. NARRATIVE: ***The narrative must address the following:***

4. Provide the rationale/justification for animal use, and discuss what alternatives (*e.g. cell lines, computer simulations, or artificial bodies*) were considered, and why the alternatives are not appropriate for this study's objective(s).

Because of ethical reasons, the proposed study of estrogen treatment and biopsy to study insulin physiology in baboons cannot be conducted in humans. Since rodents exhibit a very different pattern of estrogen secretion and levels during their abbreviated 4-day estrous cycle, rodents and other non-primate species would not provide sound models for the conduct of this particular study of the role of ovarian estrogens on insulin secretion and signaling. Moreover, *in vitro* studies with cell lines or computer simulation modeling would not permit the testing of cause and effect regulatory relationships between ovarian estrogen secretion during the menstrual cycle and pancreatic insulin secretion and target cell insulin responsivity. Consequently, the latter alternative approaches would not advance understanding of the mechanisms underlying the role of estrogen in promoting insulin secretion and action in the human.

5. Discuss the appropriateness of the species (and the animal strain, if applicable) chosen to meet the objective(s) of the study.

In the present study, we propose to continue to use the baboon as a nonhuman primate model to study the impact of the secretion of ovarian estrogen during the course of the 14-16-day proliferative phase of the menstrual cycle. Non-primate species cannot be used because they do not exhibit menstrual cycles with the relatively long-term (i.e. 14+ days) proliferative phase secretion of estrogen to investigate the gender-specific (i.e. female) effects of estrogen on insulin secretion and action. The baboon provides a scientifically valid model for the study of estrogen and insulin biology. The experience and substantial baseline data that our laboratory have with the use of the baboon further show the appropriateness of this species as a translation nonhuman primate experimental model.

6. Describe steps taken to reduce the number of animals in the study (*e.g. replacement with in vitro procedures, refinement of experimental design, refinement of procedural techniques*).

Research naïve baboons will be obtained from the Southwest National Primate Research Center for the conduct of this study. To reduce the number of animals required to obtain statistically significant and scientifically valid results, a longitudinal design will be employed in which each animal will pass through each of the different phases/treatment regiments.

7. Will the animals be subjected to procedures that may cause more than momentary or slight pain or distress? **NOTE:** *These procedures include environmental, nutritional, or behavioral modifications that increase stress, as well as chronic food or water deprivation.*

X YES (***A database search is required.*** *Complete* ***Question 8)***

___ NO (*Skip to* ***Question 9***)

8. If alternative procedures have been identified, describe the procedures below, and explain why they are not scientifically appropriate for this research project.

Alternative procedures were not identified and while in vitro studies are helpful, by themselves they do not permit testing cause: effect or applicability to the in vivo situation. Thus, we have not identified an approach including in vitro methods or cell lines that will allow us to determine the role of estrogen on insulin action and pancreatic insulin secretion.

9. Is the proposed study duplicative of research previously undertaken by the investigator or other scientists? **If yes, describe the duplicative nature of this project, and offer scientific justification.**

No

10. Federal regulations require a written rationale/justification for the number of animals to be used. Describe the statistical test (*e.g. power analyses and/or other rationales such as tissue collection needs and breeding efficiency*) used to determine the number of animals required to complete the proposed study, and provide the results of the test. **NOTE: The IACUC may require a consultation with a statistician.**

A major goal of the study is to elucidate the role of estrogen on insulin secretion and action and thus glucose uptake in key tissues. To accomplish this goal, studies will be performed in a total of 30 female baboons during an intact menstrual cycle and following ovariectomy and chronic treatment with estradiol, to mimic the follicular phase of the menstrual cycle. To elucidate the mechanisms of estrogen action and confirm that estrogen is acting directly and not indirectly, sampling will be performed in these same ovariectomized animals at critical times after an acute injection of estradiol (n = 10 animals), injection of estradiol and glucose (n = 10 animals) or injection of glucose (n = 10 animals). Assuming the populations are normally distributed (as outlined in Daniel Biostatistics: A Foundation Analysis in the Health Sciences, 4th Ed., 1987) and a pooled estimate of variance (Ø) of 2.0, 10 animals per group will be required to achieve approximately 80% power to identify differences ($P < 0.05$) between the 3 treatment groups as well as to ascertain differences in parameters during an intact cycle, following ovariectomy alone and following chronic treatment with estrogen. Thus, the number of animals has been kept to the absolute minimum necessary to obtain statistically significant and scientifically meaningful data.

D. USDA PAIN CODES:

11. For each of the appropriate pain code descriptions, list the species (and the animal strain, if applicable) and the number of animals to be used each year. **Please provide the total for all three years.**

Level B				
Breeding or holding colony protocols where animals do not undergo any manipulation.				
Species	**Year 1**	**Year 2**	**Year 3**	**Total**
Level C				
Teaching, research, experiments, or tests conducted on animals involving no or momentary/slight pain or distress (*i.e., euthanizing animals for tissues; injections; observation under normal conditions; positive reward projects; use of Acepromazine for vasodilatation in rabbits*) and **for which no pain-relieving drugs are used.**				
Species	**Year 1**	**Year 2**	**Year 3**	**Total**
Level D				
Teaching, research, experiments, surgery, or tests conducted on animals involving a degree of pain or distress (*i.e., non-survival surgery; survival surgery; antibody production; subcutaneous implants; induced infections*) and **for which appropriate anesthetic, analgesic, or tranquilizing drugs are used to relieve pain and distress.**				
Species	**Year 1**	**Year 2**	**Year 3**	**Total**
Baboon (*Papio anubis; cynocephalus*)	10	10	10	30

Level E				
Teaching, research, experiments, surgery or tests conducted on animals involving a degree of pain or distress and **for which the appropriate anesthetic, analgesic or tranquilizing drugs are NOT used because their use will adversely affect the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests**. (**SCIENTIFIC JUSTIFICATION IS REQUIRED.**)				
Species	**Year 1**	**Year 2**	**Year 3**	**Total**

E. STUDY PROCEDURES:

12. Please indicate all procedures that will be performed in this study. **Attach all required forms.**

X Non-Survival Surgery *(Complete **Attachment E, ANIMAL SURGERY**)*

____ Single Major or Minor Survival Surgery *(Complete **Attachment E, ANIMAL SURGERY**)*

X Multiple Major Survival Surgery *(Complete **Attachment E, ANIMAL SURGERY**)*

____ Prolonged Restraint *(Complete **Attachment C, PROLONGED PHYSICAL RESTRAINT OR STRESS**)*

X Collection of Tissues, Cells, or Organs

____ Adverse Conditioning

____ Special Diet

____ Food/Water Deprivation *(Complete **Attachment C, PROLONGED PHYSICAL RESTRAINT OR STRESS**)*

X Use of Biohazards or Chemical Agents *(Complete **Attachment D, USE OF HAZARDOUS AGENTS**)*

____ Burns or Trauma

____ Antibody Production *(Complete **Attachment F, ANTIBODY PRODUCTION**)*

____ X-Rays or Other Radiation

____ Tumor Transplantation/Induction

____ Toxicity Testing (LD-50) *(Complete **Attachment G, DEATH AS AN ENDPOINT**)*

____ Teaching or Training Protocol *(Complete **Question 12a** below.)*

12a. If this is a teaching or training protocol, please check all that apply.

____ Undergraduate or graduate students

____ Continuing education students (M.D.)

____ Only dead animals or tissues obtained through euthanasia by the PI will be used.

____ Demonstration (PI only performing procedures)

____ Student involvement (Students performing/assisting with procedures)

____ Use within a Biomedical Sciences Course (*ID #/Name:* _____)

____ Other **(*Explain below.*)**

F. RESEARCH DESIGN:

13. In generating the research design, note that the reviewers are scientifically knowledgeable; however, they may not be experts in your specific field of study. Please provide a brief (one or two paragraphs) overview of the project design and how each experimental goal relates to the project design. The descriptions should provide a sequential overview of all procedures and should account for each subject (by experimental group). The overview should be followed by a chronological description of all experimental procedures related to the care and use of the animals. **The use of tables and flow charts to organize the procedures, numbers of animals, and schedules is recommended.** Do not paste in method sections from grant applications or journal articles. Do not include methods pertaining to *in vitro* work, unless it applies to the care and use of animals. For each animal or experimental group, provide information on the duration of each procedure (*i.e. fluid or tissue collections, methods, sites, volumes/ weights, frequencies, etc.*) and the total time from initial contact to completion. **Although procedures involving drug manipulations and surgery are detailed in other sections of this form, their application in the research design should be stated here. Any procedures not covered in later sections of this form must be completely detailed in this section.**

By reading only this section of the *Initial Review Form*, the IACUC should be able to clearly determine each experiment being performed on each individual animal.

Experimental Design

A longitudinal study as shown in the figure below will be conducted to determine the effect of estrogen on insulin secretion and signaling in 30 nonpregnant baboons which will pass through the entire experimental sequence outlined (i.e. intact proliferative phase, chronic estradiol and acute estradiol following ovariectomy).

Adult female baboons weighing 10-12 kg body weight will be obtained from the Southwest National Primate Center (San Antonio, TX) and housed singly or in pairs as established by successful socialization in large primate cages located in air-conditioned rooms within the vivarium at EVMS. We attempt to socialize and pair house whenever possible. However, if non-successful socialization arises, animals will be single housed.

[1] Studies during the intact proliferative phase

The proliferative as well as the ovulatory and secretory phases of the menstrual cycle will be determined by daily recording of the pattern of perineal turgescence/deturgescence and onset of menses and confirmed by serum estradiol (E_2) and progesterone (P_4) levels. Briefly, blood samples (2-3 ml) will be obtained via a 21-gauge needle from a peripheral saphenous vein after brief restraint and sedation with ketamine HCl (5-10 mg/kg body weight im) 5-7 days prior to anticipated onset of perineal deturgescence (which is the day of ovulation) and on 5-8 days post deturgescence and the levels of E_2 and P4 determined by RIA.

[a] Intravenous Glucose Tolerance Test

An intravenous glucose tolerance test (GTT) to assess pancreatic insulin secretion/responsivity and insulin sensitivity will be performed between day 10- 13 of the proliferative phase (day 0 = onset of menses; days 14-16 = ovulation, length of menstrual cycle approximates 32 days) of the subsequent menstrual cycle and when serum E_2 levels are elevated (approximately 120-150 pg/ml). Briefly, the day before experimentation, baboons will be sedated with ketamine-HCl (5-10 mg/kg BW) and a 3 ml blood sample obtained via a 21-gauge needle from a peripheral saphenous vein and serum E_2 determined by rapid RIA to confirm that the animal is in pre-ovulatory/late proliferative phase of the menstrual cycle. Baboons will be fasted overnight and the following morning briefly restrained and sedated with ketamine, HCl (5-10 mg/kg BW, im) and catheters inserted into a peripheral saphenous vein (19 gauge x 24 inch, sterile) and antecubital vein (22 gauge, 1 inch) and a 250 ml 0.9% sterile saline drip with 500 mg ketamine initiated via the saphenous vein. The saphenous line will be flushed by drawing 1 ml prior to each blood sample. A base line 3 ml blood sample will be collected and blood gases (pO_2; pCO_2), pH, chemistries (Na, K, Cl and glucose) determined using an iSTAT portable clinical analyzer (Abbot Labs, East Windsor, NJ). A bolus injection of dextrose (0.25 g/kg BW) will be administered via the antecubital vein at experimental time 0 min and blood samples (3.0 ml each) obtained via the peripheral saphenous vein at 0, 1, 3, 5, 10, 20, 40, 60 and 90 min after dextrose administration. During the experiment the blood pressure, heart rate, and respiration rate are monitored and body temperature maintained via a circulating water pad and/or heated table. Immediately after the 90 min blood sample is obtained, catheters are removed and the animal is given 100 mg (1 ml) iron dextran im, returned to the housing cage and monitored to recovery. Glucose levels will be quantified via an iStat portable clinical analyzer (Abbot Labs, East Windsor, NJ) on 0.1 ml blood and plasma insulin levels assessed by solid-phase chemiluminescent immunometric assay (Siemens Healthcare Diagnostics, Tarrytown, NY), which displays a sensitivity of 2µ IU/ml and intra- and inter-assay coefficients of variation of 5.7% and 5.9%, respectively, for baboon insulin. The mean levels of insulin and glucose at 1 min and average at 1, 3 plus 5 min (minus baseline) will be calculated. The homeostasis model of assessment of insulin resistance (HOMA-IR), which is calculated by the fasting baseline glucose x fasting baseline insulin levels ÷ 22, conversion factor for molar units, will be employed as an index of insulin sensitivity. Extensive comparison of surrogate indices of insulin sensitivity (i.e. HOMA, Log HOMA, 1/HOMA, Quickl, and 1/ Fasting insulin) with the complex, labor, technical and cost-extensive hyperinsulinemic euglycemic glucose clamp method in rhesus monkeys has shown that each of the surrogate indices provided reliable results that were comparable to the glucose clamp method and can be used in large primates to assess insulin sensitivity

(Lee et al, 2011).

[b] Non-invasive Doppler ultrasonography: tissue biopsy before and after glucose bolus in intact proliferative phase

Following a respite of 30 days, e.g. completion of a second menstrual cycle, analysis of Microvascular Flow and Brachial-Artery Flow Mediated Dilation by non-invasive ultrasound as outlined below and procurement of tissue biopsies before and after a glucose bolus will be performed on day 10-13 of the proliferative phase of the next menstrual cycle as determined by menstrual cycle history/perineal turgescence and confirmed by serum levels of E_2. Briefly, the day before experimentation, baboons will be sedated with ketamine-HCl (5-10 mg/kg BW) and a 3 ml blood sample obtained via a 21-gauge needle from a peripheral saphenous vein and serum E_2 determined by rapid RIA. Animals will then be fasted overnight, the next morning sedated with ketamine, intubated, anesthetized with isoflurane and a 19 gauge 24 inch catheter inserted into and a blood sample (3 ml) obtained from a peripheral saphenous vein and a slow drip of 0.9% sterile saline initiated. After stabilization of heart rate, blood pressure (approximately 10 mins), Microvascular Flow (approximately 10 mins) and Brachial Artery Flow-Mediated Dilation (approximately 10 mins) will be determined as described below. Following completion of these noninvasive studies, a midline and 1-2 lateral abdominal incisions are made through the skin only and insufflation of the abdominal cavity with CO_2 gas is initiated. A trocar is used to establish 2-3 ports into the abdomen for laparoscopic camera insertion and 1-2 ports for surgical manipulation of internal organs and a biopsy (experimental time 0 min) of visceral and subcutaneous adipose (fat) will be obtained. The pancreas will be identified and a 2mm Bergstrom Stiles needle (Cadence Science, Lake Success, NY) inserted from the tail region through the center of the gland and the needle withdrawn to obtain a biopsy (thread). A biopsy of the quadriceps muscle will be obtained by making a small 10 mm incision via a #10 surgical blade in the skin and fascia to expose the underlying muscle and using a 8 mm sterile punch biopsy. Hemostasis will be attained by direct pressure applied to the biopsy sites and sealing with Gel-foam. A bolus injection of dextrose (0.25 g/kg BW) into an antecubital vein will then be made and 20 min later biopsies of visceral adipose, pancreas, and quadriceps muscle again obtained at sites at least 2 cm removed from the first biopsies. The pancreatic, adipose and skeletal muscle biopsies/tissues will be used to assess components of insulin biosynthesis, the insulin signaling pathway and angiogenesis pathway essential for vascularization. Incision sites will be closed using absorbable suture. Animals are then bilaterally ovariectomized as described below. Briefly, the ovaries are located and electrocautery used to sever the ovarian ligament and surrounding tissue and the ovaries removed. Although this laparoscopic approach should pose no problem, should there be a problem, collection of biopsies and ovariectomy will be conducted surgically following a laparotomy.

Bilateral Ovariectomy

Briefly, a midline and 1-2 lateral abdominal incisions are made through the skin only and insufflation of the abdominal cavity with CO_2 gas is initiated. A trocar is used to establish 2-3 ports into the abdomen for laparoscopic camera insertion and 1-2 ports for surgical manipulation of internal organs. The ovaries are located and the ovarian ligament and associated blood vessels of the right and left ovaries will be clamped and cauterized and both ovaries excised. The clamps will then be carefully removed, the ligament/cautery site observed for bleeding. Incisions will be closed using absorbable suture and vet-bond skin adhesive applied. If a laparotomy is required, a midline abdominal incision through the skin, fascia/muscle and peritoneum will be made and the ovaries localized and a retractor and lap sponge used to clear the field if needed. The ligaments and associated blood vessels of the right and left ovaries will be clamped and cauterized and both ovaries excised. The clamps will then be carefully removed, the site observed for absence of bleeding and the abdominal cavity rinsed with sterile saline. The peritoneum, sc fascia and skin will then be closed separately with absorbable suture. Vet-bond skin adhesive will be applied to the incision site once closed. Isoflurane will then be shut off and the animal monitored until a swallowing reflex is present. The animal will then be extubated and continued to be monitored in home cage until fully upright and responsive to stimuli. Post-operative monitoring will be performed by Comparative Medicine staff using procedures outlined in the EVMS Post-Operative Care Guidelines Manual and in Attachment E below. Animals will be treated post-operatively with analgesics and recovery monitored as outline in Attachment E below.

Non-Invasive Doppler Ultrasonography Procedures

[a] Microvascular Volume Flow:

Microvessel volume flow rate will be quantified by pulsed-color 3D Doppler ultrasonography by placement of an 8-MHz transducer attached to GE Voluson ultrasound system over the inner surface of the wrist. Microvessel volume flow rate will be quantified by the formula Q_A (ml/min) = V (time-averaged mean velocity in cm/sec) x πr^2 (cross sectional area of vessel, r = radius of an average of 4 microvessels, x 60 sec/min). Three separate flow rates will be obtained over a 5-min period and the results averaged.

[b] Brachial Artery Flow-Mediated Dilation

Vascular endothelial function will be assessed indirectly by brachial artery flow-mediated dilation, an established noninvasive measure used in humans. Brachial artery flow will be determined immediately after microvessel flow assessment and immediately before laparotomy in intact, proliferative phase, chronic estradiol-treated and ovariectomized animals. An 8-MHz transducer and GE Voluson ultrasound system will be used to capture flow images and assess maximum brachial artery diameter and volume flow during diastole. Following

baseline Doppler recordings, a standard cuff will be positioned around the arm approximately 1-2 inches below the antecubital fossa and inflated to 50 mmHg above maternal systolic blood pressure (i.e. average systolic blood pressure in isoflurane anesthetized baboons approximates 60 mmHg) for 5 min to occlude brachial artery flow and induce hyperemia. Following cuff deflation, brachial artery flow images are recaptured continuously for 2 min in diastole (gated with ECG R wave) or a total of 4 separate measurements over an 8 min period. Maximum vessel diameter is determined from Doppler image and the % flow-mediated dilation = maximum diameter – baseline diameter % baseline diameter x 100.

[2] Studies during Chronic E_2 treatment

Chronic E_2 model

Baboons will be left for 8-10 weeks after ovariectomy to ensure metabolic clearance of ovarian steroid hormones and complete recovery from the surgery. Animals will then be administered 17β-E_2 via silastic implants in an amount which will replicate the elevated levels observed during the late proliferative phase of the normal menstrual cycle (i.e. approximately 120-150 pg/ml). Baboons will be sedated with ketamine HCl and a peripheral saphenous blood sample (3 ml) obtained for analysis of serum E_2. Hair will be removed from the site of implantation (abdomen or subscapular region) and the animals lightly anesthetized with isoflurane administered via nose cone or a ketamine/xylazine cocktail to reduce the number of times the animals are exposed to a general anesthetic. A sterile disposable drape is placed over the area and using aseptic technique, an 0.8-1.0 cm incision will be made in the abdominal skin or subscapular region and a sterile silastic implant 3-6 cm long and 0.5 cm diameter containing 17β-E_2 inserted and the skin closed with absorbable suture. Animals will be returned to their home cages and 7 days later the animal will be sedated with ketamine (5-10 mg/kg BW), a peripheral saphenous blood sample (2-3 ml) obtained via a 21-gauge needle, the first E_2 implant removed and 2 new silastic implants containing E_2 inserted sc in the same region approximately 2-3cm from the 1st implant using the same aseptic procedures. Following the second implant, on day 2,3,4 and 5 blood samples (2-3 ml) will be obtained from a peripheral saphenous vein via a 21-gauge needle after brief restraint and ketamine sedation (5-10mg/kg) and serum E_2 levels measured by RIA to ensure that the targeted level of E_2 has been obtained. The length of silastic capsules will be decreased or increased to achieve the correct level of E_2. As an alternative to use of silastic implants, which involves isoflurane anesthetization, a preliminary study will be conducted to assess possible efficacy of daily injection of estradiol benzoate (which has a 24h half life) to maintain chronic elevations of estrogen in ovariectomized baboons. Baboons would be briefly sedated with ketamine and E_2 benzoate administered via sc injection with 21 gauge needle in increasing doses every 14 days (beginning at approximately 1-10 µg/kg BW in 0.50 ml sesame oil and increasing to maximum of 10-100 µg/kg BW over the 14 day period) to achieve levels which approximate the late proliferative phase (~120-150 pg/ml). If the daily injection approach is effective it will become the standard method for this study.

Glucose Tolerance Test in Chronic E2 model:

Around day 14 of treatment with chronic E_2, baboons will be sedated with ketamine and an iv GTT performed and blood samples (3 ml) obtained over a 90 min period (0, 1, 3, 5, 10, 20, 40, 60 and 90 min) as described previously. At the end of the GTT, the previous silastic implants will be removed and 2 new E_2 implants inserted sc in a different area of the same region using the above aseptic technique.

Non-invasive Doppler Studies and tissue biopsies before and after glucose bolus in chronic E2 model:

Seven to fourteen days later the animals will be sedated with ketamine, anesthetized with isoflurane and a 19 gauge 24 inch catheter inserted into a peripheral saphenous vein and a slow drip of 0.9% sterile saline initiated. After stabilization of heart rate, blood pressure (approximately 10 mins), Microvascular Flow (approximately 10 mins) and Brachial Artery Flow-Mediated Dilation (approximately 10 mins) will be determined as described above. Following completion of these studies, biopsies of the pancreas and a biopsy (thread) visceral and subcutaneous adipose (fat) will be obtained. The pancreas will be isolated and a biopsy obtained using a 2 mm Bergstrom Stiles needle as outlined in the above laparoscopic approach (1b). A biopsy of the quadriceps muscle will be obtained by making a small incision in the skin and fascia to expose the underlying muscle and using a 8 mm sterile punch biopsy. Hemostasis will be achieved by direct pressure to and sealing of the biopsy sites with Gel-foam. A bolus injection (21 gauge or smaller bore needle) of dextrose (0.25 g/kg BW) into an antecubital vein will then be made and 20 min later biopsies of visceral adipose, pancreas, and quadriceps muscle again obtained at sites at least 2 cm removed from the first biopsies. The E_2 silastic implants will then be removed, the abdomen sutured closed and animals treated with analgesics, monitored and returned to their home cage as described above. Although this laparoscopic approach should pose no problem, should there be a problem, collection of biopsies and ovariectomy will be conducted surgically following a laparotomy.

[3] Studies during Acute E_2 treatment

Acute E_2 Model

After the previous treatment regiment, baboons will be left untreated for 8-10 weeks to allow metabolic clearance of E_2 and complete recovery from surgical intervention. Baboons will then be treated daily for 5 days with the aromatase inhibitor Letrozole (35 µg/kg BW, sc via a 21 gauge needle) to block potential peripheral aromatization of androgens to estrogen and thus ensure complete estrogen suppression.

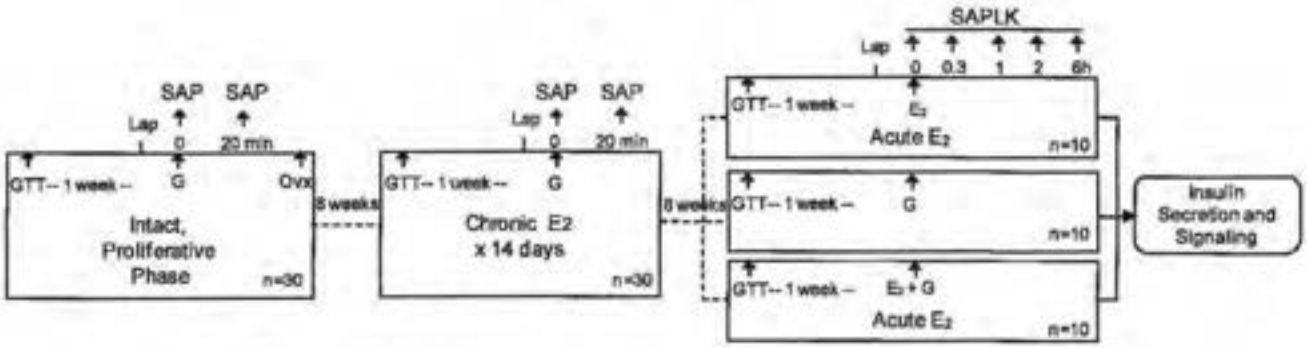
Glucose Tolerance Test in Ovariectomized Acute E2 model:

Letrozole-treated ovariectomized baboons will then be fasted overnight, sedated with ketamine, catheters

placed into antecubital and saphenous veins and an iv GTT performed essentially as described above to assess impact of estrogen deprivation on insulin secretion/signaling. Briefly, a bolus injection of dextrose (0.25 g/kg BW) will be administered via the antecubital vein at experimental time 0 min and blood samples (3.0 ml each) obtained via the saphenous vein at 0, 1, 3, 5, 10, 20, 40, 60 and 90 min after dextrose administration.

One week later, baboons will be fasted overnight and on the following morning sedated with ketamine, anesthetized with isoflurane and catheters placed into antecubital and saphenous veins as described above. Animals are then divided into 3 groups; group 1 will receive a bolus of estradiol (17β-E_2; 0.10 - 0.25 µg/kg BW in 1.0 ml 5% ethanol/saline; group 2 will receive a bolus of dextrose (0.25 g/kg BW; in 1.0 ml sterile water) and group 3 will receive a bolus of 17β-E_2 and dextrose (doses as above) administered over a 1 minute period via the antecubital vein catheter. The dose of estradiol is calculated (but may need to be adjusted) to achieve a serum estradiol level of approximately 150 pg/ml within 5 min. A midline incision in the abdomen will then be made and biopsies of visceral adipose, pancreas and skeletal muscle obtained exactly as described above before (i.e. experimental time 0) and at 0.3, 1, 2, and 6 h after E_2 and/or glucose administration. The needle biopsies will be obtained in different areas of the tissue. Blood samples (3 ml) will also be obtained from the saphenous vein at each of the biopsy times. A biopsy will also be obtained from the liver and kidney at 6h after E_2 and/or glucose and the animals immediately euthanized with Euthasol (100 mg/kg BW iv).

EVMS PPG - Estrogen Regulation of Pancreatic Beta Cell Function/Insulin Secretion and Insulin Signaling Mechanisms in the Nonpregnant Baboon



Treatments*

GTT – Glucose tolerance test (dextrose iv bolus, ketamine sedation)
Lap – Laparotomy (isoflurane anesthesia)
G – Glucose (i.e. dextrose) iv bolus
SAP – Skeletal muscle, visceral adipose, pancreas biopsy
L – Liver biopsy (6 h only)
K - Kidney biopsy (6 h only)
Ovx – Ovariectomy
E_2 – 17ß-Estradiol

* Longitudinal study (i.e. single baboon passes through entire sequence; n=30 baboons)

Anatomical and molecular analyses

- Structure (histology)
- Pancreatic insulin biosynthesis (proinsulin convertases-1, -2, -3, insulin and C-peptide mRNA and protein)
- Insulin signaling (GLUT-2/-4, insulin receptor, phosphorylated IRS-1/-2, PI3K, protein kinase B (Akt) glycogen synthase kinase (GSK) -3 α/ß, mammalian target of rapamycin (MTOR), cell glucose uptake)
- Estrogen receptor α and ß expression
- Oxidative stress /apoptosis (ROS, SOD, MAPK, caspase-3, TUNEL)
- Angiogenesis (VEGF, Ang-1/-2, microvessel TJ and blood flow)
- Vascular protection (NOS, HIF, ET-1)
- Inflammation (TNFα, cytokines)
- *In vitro* studies in pancreas, skeletal muscle, adipose, liver at end of final *in vivo* studies

Physiological analyses

- Vasodilation via brachial artery flow
- Microvessel flow
- Skeletal muscle capillarization

14. **Adverse Effects: Monitoring and Management:**

14a. In detail, describe the possible adverse effects for each experimental procedure and/or agent administered to animals. For each item, include a statement detailing how the adverse effects will be clinically managed, should they occur.

Isoflourane: Anesthetic agent during surgical procedures. Can cause depreciated BP, respiratory depression, hypotension and arrhythmias.

Monitoring: CompMed staff will monitor for all these physiological parameters and treat accordingly.

Ketamine: Used for sedation for blood draw can cause tenderness at injection site, depreciated appetite, bruising at site of blood draw and an increased tolerance to ketamine.

Monitoring: Animal will be injected in various areas of the rump, alternating legs used to draw blood, proper technique used to draw blood, lowest possible dose of ketamine administered and food enrichment will be added to stimulate a depreciated appetite.

Estradiol: Used to increase estrogen levels. At the doses proposed there should be no adverse physiological effects. Irritation at the site of silastic implants or sc injection may occur.

Monitoring: Maintain aseptic techniques and alternate areas of implant insertion to lower the possibility of irritation.

Ovariectomy: General risks include blood loss, infection and abdominal adhesions.

Monitoring: Aseptic technique used to reduce infection rate. Animals are monitored during procedure and all vitals are recorded on anesthesia sheet and maintained in the animal record. Any adverse events will be addressed by Comp Med clinical staff and veterinarian.

Flunixin meglumine (Banamine): IM injection administered for pain management. May cause GI upset if given for to long or overdose.

Monitoring: Treatment is administered as directed by facility veterinarian, any sign of GI upset will be noted and staff members contacted.

Dextrose: At the standard dose to perform glucose tolerance test there should be no adverse effects.

Biopsy of skeletal muscle, visceral adipose and pancreas: Bleeding may occur at the site of biopsy (via Bergstrom Stiles needle).

Monitoring: Hemostasis will be attained by direct pressure with sterile gauze and sealing with Gel-foam.

Letrozole: Used to suppress estrogen production. At the dose proposed there will be no adverse effects. Irritation at the injection site may occur.

14b. Describe the clinical parameters that will be monitored to indicate adverse effects, pain, and/or distress to animals. The parameters should be specific to the species and to the procedure(s). Include the frequency of monitoring throughout the study.

The overall health and behavior of baboons will be assessed by PI staff. Comp Med staff will also perform routine daily health checks of all baboons. Staff will look for chronic loss of appetite, diarrhea, swelling at point of interest, decreased activity and alertness and overall behavioral changes. Animals will be monitored 2X a day on day 1-3 and once a day for day 4-7 post-operatively. All observations will be recorded on Post-op monitoring sheet and maintained in the animal records kept in the animal facility.

14c. What conditions and/ or complications will lead to removal of an animal from the study (*i.e. an early endpoint*)?

Excessive blood loss or inability to fully recover (e.g. animal appears lethargic; loss of appetite; complications with incision site) from abdominal surgery. If an animal experiences >20% weight loss from initial (or any) procedure, the animal will be removed from the study. The veterinarian will be consulted on any animals with observed long term adverse affects.

G. ADMINISTRATION OF ANESTHESIA, THERAPEUTICS, AND EXPERIMENTAL AGENTS:

15. Indicate the sedatives/tranquilizers, anesthetics, analgesics, antibiotics, and other relief agents that will be administered. If no anesthetics, analgesics, or other pain relief methods will be used, please provide a strong justification for withholding analgesic agents in Question 15a below. The withholding of analgesic agents must be based upon cited scientific fact or provided experimental data. **NOTE: Some anesthetics and analgesics are controlled substances and require Virginia Board of Pharmacy and DEA licenses for purchase and use.** ADD ADDITIONAL ROWS AS NEEDED.

	Dose (mg/kg)	Route	Frequency	
Sedatives/Tranquilizers				
Ketamine	5-10 mg/kg	IM	For blood collection and for surgical preparation	
Xylazine	6mg/kg	IM	For silastic capsule implant	
Anesthetics – General				
Isoflurane	~2% MAC	Inhalant	At surgery and for implantation of silastic capsules	
Anesthetics – Local				
Analgesics			**Frequency**	**Length of Administration**
Flunixin meglumine (Banamine)	2mg/kg	IM	At surgery and 4-8 h following	Surgery + 2 days BID
Antibiotics				
Miscellaneous				
Iron Dextran	100mg	IM	Once after every procedure (GTT, capsule implant, surgery)	Once
Children's Chewable vitamins with iron	One tablet	PO	Daily	Throughout the study

15a **JUSTIFICATION FOR WITHHOLDING ANALGESIC AGENTS**

16. Will agents other than anesthetics or analgesics (*i e drugs, reagents, cells, etc*) be administered?

X YES (*Complete **Question 17** for each agent. Add additional sections as needed.*) _____ NO (*Skip to **Question 18***)

17 Agent: Letrozole Agent vehicle: Sesame oil

Route/site: sc Volume per administration: 0.2ml

Frequency of administration: Daily x5 days, this drug is administered (35 µg/kg BW/ day) to prevent estrogen formation in fat and thus ensure complete removal of estrogen from the body.

List all expected side effects and/or changes in the animal's behavior:

Irritation at injection site

Agent: Dextrose *iv* *purchased as such or diluted in sterile water as necessary*

Route/site: iv Volume per administration: 0.25g/kg

Frequency of administration: As a bolus iv injection to conduct glucose tolerance test and at surgery

List all expected side effects and/or changes in the animal's behavior:

There are no expected side effects or changes in animal behavior from this dose of dextrose.

Agent: Estradiol Agent vehicle: Silastic capsule (sc)/sesame oil injection (sc)/saline/5% ethanol saline (iv)

Route/site: sc or iv Volume per administration: 0.5 ml (sc) 1.0 ml (iv)

Frequency of administration: Estradiol silastic implants (3x over ~30 days); estradiol benzoate sc (daily x 14 days); estradiol iv (once as bolus)

List all expected side effects and/or changes in the animal's behavior:

There are no expected side effects or changes in animal behavior from estradiol at these doses.

NOTE: Your signature on page 4 certifies that all drugs used on animals before, during, or after an experimental or surgical procedure will be obtained from legal sources, will be pharmaceutical-grade, unless otherwise approved, and will be disposed of properly when out-of-date or no longer needed. **All controlled substances MUST be kept in a double-locked compartment, and records documenting each use of a controlled substance MUST be maintained.**

H. SPECIES SELECTION AND ORDERING:

18. Please indicate the species and the number of animals requested.

Species (Common Name & Strain)	Total Number Requested for a 3-Year Period	Avg. # to be Maintained in Animal Facility	Max. # to be Maintained in Animal Facility
Papio anubis (baboon)	30	15	15

If the project involves NON-HUMAN PRIMATES, complete *Attachment B, NON-HUMAN PRIMATE ENHANCEMENT PROCEDURE.*

19. Will animals be ordered through the Division of Comparative Medicine?

X YES _____ NO (***Identify the source and provide the rationale/justification.)***

20. Will the animals require special housing (*e.g., specific bedding requirements. isolator cages, special feed or handling, etc.*)?

_____ YES (***Describe all special requirements.***) X NO

21. Does this study involve the use of animals that will be maintained as a colony over a long period of time? ***(Colony is defined as "breeding or holding of animals for reuse in other experiments.")***

X YES (*Complete* ***Questions 21a and 21b***) _____ NO (*Skip to* ***Question 22***)

21a. List the number of new animals you are planning to purchase for the colony: 24

21b. List the number of animals you are planning to use from an existing colony: 6

I. PERSONNEL TRAINING:

22.	In Section 1, list the name of each person involved with the project, along with the species to be used, the person's years of experience with that species, and the person's training information. In Section 2, continuing with the column from Section 1, note each person's functional role for each species listed. **ADD COLUMNS OR PAGES AS NEEDED**

SECTION 1: PERSONNEL INFORMATION						
NAME:	Gerald Pepe	[redacted]	[redacted]			
(Species / Experience) Species used in project / Years of experience with the species listed	Species / Exp	Species / Exp	Species / Exp	Species/Exp	Species/Exp	Species/Exp
	P. anubis / 38	*P. anubis* / 12	*P. anubis* / 16			/
	/	/	/	/	/	/
	/	/	/	/	/	/
Occupational Health and Safety (OHSP) Training Certification Number	[redacted]	[redacted]	[redacted]			
Occupational Health and Safety Risk/Health Assessment Date (Month / Year)	7/24/00	3/8/04	7/24/00			
LATA training by species	[redacted] Nonhuman primate	[redacted] Nonhuman primate	[redacted] Nonhuman primate			
SECTION 2: FUNCTIONAL ROLE FOR EACH SPECIES LISTED						
Supervision	X	x				
Care and Handing	X	x	x			
Anesthesia		x				
Surgery	X	x	x			
Post-Surgical Care		x				
Monitoring		x	x			
Euthanasia	X	x				

22a. Provide information regarding the degree of training and procedural experience for each individual listed in Question 22.

Dr. Pepe has performed surgeries on baboons for more than 30 years. His team works closely with him to provide for collection of tissues during surgery. [redacted] has a wealth of experience working with these animals and is experienced in intubation/surgical preparation and surgical assistance. Moreover, [redacted] has been trained by Dr. Pepe and has been performing surgeries (cesarean section) without direct assistance of Dr. Pepe (e.g. Dr. Pepe has not scrubbed in but is available or on site) for over 8 years. [redacted] has significant experience assisting Dr. Pepe in the conduct of the surgical experimentation and to collect tissue samples.

22b. List any person that will require supplemental training from the Division of Comparative Medicine, and describe the desired training.

Dr. Pepe and [redacted] would like assistance with the procedure from the attending Vet and / or consultation from attending physician of Sentara General.

J. ANIMAL USE PROCEDURES (EXCEPT SURGICAL PROCEDURES):

ALL SURGICAL PROCEDURES MUST BE DETAILED IN *ATTACHMENT E, ANIMAL SURGICAL PROCEDURES*

23. Will cells, tissues, and/or organs be collected?

X YES (*Complete all applicable sections below.*) _____ NO (*Skip to **Question 24***)

23a. **Blood sampling**

Technique:	Blood samples (2-3ml) are collected prior to and following ovariectomy and during estradiol treatment. Baboons are sedated with ketamine HCl and a blood sample obtained from a saphenous vein using a 21 or 23 gauge needle. Area is cleaned with alcohol pad prior to blood draw. Serum is stored at -20C and subsequently analyzed for estradiol. Blood samples (3.0 ml) will also be collected via saphenous vein catheter during glucose tolerance test (GTT)
Sample site:	Saphenous vein
Volume per sample:	1-3 ml
Frequency & duration of sampling:	Daily for 5-7 days in one menstrual cycle. Daily during the 14 day chronic ovariectomy study. During GTT and at timed intervals during surgical biopsy procedure.

23b. **Urine/feces sampling**

Method: _____

Frequency & duration of sampling: _____

23c. **Collection of tissues**

Tissues collected: During surgery: Skeletal muscle, visceral adipose and pancreas via biopsy
Following euthanasia: Liver, kidneys and other organs or tissues of interest.

When collected (before or after euthanasia): Both

Disposition of collected tissues: All tissues utilized for research experiments. The carcass will be properly disposed of by Comp Med

24. Will behavioral testing be conducted?

X No behavioral testing will be conducted.

_____ Yes, behavioral testing will be conducted with significant restraint or noxious stimuli. (*Complete **Attachment C, PROLONGED PHYSICAL RESTRAINT OR STRESS***)

_____ Yes, behavioral testing will be conducted without significant restraint or noxious stimuli. (***Describe the procedure below.***)

25. Will a special diet be required?

_____ YES (*Complete **Questions 25a-25c***) __X__ NO (*Skip to **Question 26***)

25a. Describe the anticipated nutritional deficit or supplementation.

25b. Provide the reason(s) for and treatment of the deficit or supplementation.

25c. How often will the animals be weighed? _____

How much weight change will be permitted before the animal will be removed from the study? _____

26. Will indwelling catheters or implants be used?

X YES *(Complete a section below for each site. Add additional sections as needed.)* _____ NO *(Skip to **Question 27**)*

26a(1) Site: Insertion of estradiol containing silastic capsules sc in lower quadrant of abdomen or scapular region.

Type & Size: Silastic capsules: 3-6 cm length x 0.5 cm diameter

Maintenance: Incision site kept clean and topical antibiotic applied as needed. Capsules are changed out 7-14 day interval Duration: ~30 days

26a(2) Site: _____

Type & Size: _____

Maintenance: _____ Duration: _____

27. Will tumors be transplanted or induced?

_____ YES *(Complete a section below for each site. Add more sections as needed.)* x NO *(Skip to **Question 28**)*

27a(1) Transplant or Induction Site. _____

Anticipated Functional Deficit(s) and Management. _____

Maintenance. _____ Duration: _____

27a(2) Transplant or Induction Site. _____

Anticipated Functional Deficit(s) and Management: _____

Maintenance: ___ Duration: _____

K. ANIMAL CARE:

28. Describe, in detail, the plans for medical care of the animals in the proposed study, and **identify, by name and job classification**, the responsible person(s) on the investigative staff. **NOTE:** ***Routine observation of the animals and medical intervention is the responsibility of the principal investigator.***

[redacted], Research and Animal Technician; will monitor the animals routinely in conjunction with CompMed for normal behavior. Animals on protocol study will be monitored for stress or surgical / procedural complications as outlined in the IACUC protocol and Comp Med Post-op care guidelines manual. Any observed deviation in normal behavior or physical state will be brought to the attention of Comp Med staff and facility veterinarian.

29. Will this study require special observation?

_____ YES *(Complete **Question 29a**)* x NO *(Skip to **Question 30**)*

29a. Frequency of Observation: _____

By whom *(Identify by Name)*: _____

Starting: _____ Ending: _____

30. Indicate any special instructions that should be observed for animals found dead (*e.g., call investigator, refrigerate or freeze carcass, disposal, etc.*). **If you would like for the Institutional Veterinarian to necropsy animals that die unexpectedly, please indicate how you would like for the tissues to be handled.**

PI staff should be notified immediately. Contact numbers are available to Comp Med staff. Collection of sections of pancreas, skeletal muscle, visceral adipose, kidney, liver, and lung should be placed in fixative (10% buffered formalin)

L. DISPOSITION OF ANIMALS:

31. Please indicate the method(s) of animal disposition (***Check all that apply***):

X Euthanasia *(Complete **Questions 33a-33c**)*

_____ Death as an Endpoint *(Complete **Attachment G, DEATH AS AN ENDPOINT**)*

X Return to animal colony

_____ Available for transfer into another EVMS IACUC protocol*

_____ Available for transfer to another research institution*

_____ Available for adoption as a companion

_____ May be culled for tissue sharing

_____ Other (*Explain*): _____

* ***Animals that have undergone survival surgery in one IACUC protocol may not be transferred to another survival surgical protocol, unless the request is specifically reviewed and approved by the IACUC. These animals may be transferred to non-surgical or non-survival protocols without IACUC review.***

32 Disposition of Surviving Animals

32a. Will animals survive the protocol/procedures?

X YES *(Complete **Question 32b**)* X NO *(Skip to **Question 33**)*

32b Will animals survive without harm or disability?

____ YES *(Skip to **Question 33**)* X NO *(Complete **Question 32c**)*

32c Describe the harm or disability and the plans for management of the disability.

Animals will be euthanized at completion of the study. Animals will undergo a bilateral ovariectomy and be infertile.

33. Euthanasia

33a. Will the animals be euthanized?

X YES *(Complete **Questions 33b-33d**)* ____ NO *(Skip to **Question 34**)*

33b. Explain why the animals will be euthanized.

The animals will have undergone 2 survival surgeries for purposes of ovariectomy and then tissue biopsy.

33c Indicate how the animals will be euthanized.

Euthanasia agent/procedure: Pentobarbital (fatal plus solution)

Dose: 100mg/kg BW Route: iv

33d Per the *AVMA (American Veterinary Medical Association) Guidelines on Euthanasia*, June 2007 (formerly the *2000 Report of the AVMA Panel on Euthanasia*), most physical methods of euthanasia, when done appropriately, are "conditionally acceptable," meaning that the nature of the techniques may not consistently produce humane death or they present a greater potential for operator error or safety hazards. In those situations where physical methods may be the most appropriate method for euthanasia and rapid relief of pain and suffering, extreme care and caution must be exercised, and personnel performing physical methods of euthanasia must be well trained and monitored for each type of physical technique. **If a physical method, such as decapitation or cervical dislocation, will be used as the primary means of euthanasia, please provide scientific justification.**

N/A

M. ANIMALS BROUGHT INTO AND TAKEN OUTSIDE OF THE ANIMAL FACILITY:

34 Will any animals be transferred into the EVMS Animal Facility from another institution?

X YES *(Complete Questions **34a-34b**)* ____ NO *(Skip to **Question 35**)*

All animals received from other than approved vendors must undergo a quarantine period to allow for evaluation of the health status of the animals prior to their introduction into the colony. They may also require testing and segregation to determine their health status.

THE PRINCIPAL INVESTIGATOR SHOULD DISCUSS THESE ISSUES WITH THE DIVISION OF COMPARATIVE MEDICINE PRIOR TO INITIATING ANIMAL TRANSFER. THE PRINCIPAL INVESTIGATOR IS RESPONSIBLE FOR ALL RELATED CHARGES.

34a. How long will the quarantine or stabilization period last?

As determined by EVMS Comp Med guidelines-generally 6-8 weeks or three negative TB results.

34b. How long will the animals be housed at EVMS?

Up to 3 years

35. Will the animals be taken out of the Central Animal Facility (*i.e. CompMed, [redacted] floor*) for any reason (*i.e. manipulation, surgery, temporary housing, etc.*)?

_____ YES *(Complete **Questions 35a-35c**)* X NO *(Skip to **Question 36**)*

35a. To what building(s) and room(s) will the animals be taken? *(Indicate what procedure(s) will be performed in each specific location.)*

35b. How will the animals be transported? (*Be specific. Include all safety precautions for animals and personnel.*)

35c. How often will the animals be taken to the location(s) listed above, and for what duration of time per incident?

36. Will the animals be used or housed in locations outside of the Central Animal Facility (*i.e. CompMed [redacted] floor*) for periods greater than 12 hours?

_____ YES *(Complete **Questions 36a-36c**)** X NO

The location must be certified as a satellite-care facility and undergo a semi-annual inspection by the IACUC.

36a. In what building(s) and room(s) will the animals be used or housed?

36b. Describe the animal husbandry to be performed, and identify, by name, the person(s) who will provide husbandry.

36c. How long will the animals be used or housed in the satellite-care facility?

Eastern Virginia Medical School
Institutional Animal Care and Use Committee

Attachment B: Nonhuman Primate Enhancement Procedure

Project Title: Estrogen regulation of insulin secretion and signaling in the non-pregnant baboon

1. Paired housing: Nonhuman primates used under this protocol can be housed in the same primary enclosure with one or more compatible primates.

   X YES (*Skip to Question 2*) _____ NO (*Complete Question 1a*)

   1a. Justify why the animal must be singly housed:

Animals do not need to be housed singly. The only time that an animal would need to be housed alone is during post-op recovery or in the event that a compatible partner cannot be established. The latter would be documented in the animal record and occur only with agreement of the attending veterinarian. Thus, we will socialize all animals and pair house animals in large primate cages whenever possible. If non-successful socialization arises, animals will be housed singly but in rooms with other baboons.

2. Nonhuman primates used under this protocol will be provided with a variety of devices as described in the EVMS Primate Enhancement Program (this can be provided to you by the Office of Research or the Division of Comparative Medicine (CompMed) upon request).

   X YES _____ NO (*justify in the space below*):

Eastern Virginia Medical School
Institutional Animal Care and Use Committee

Attachment D: Use of Hazardous Agents

Project Title: Estrogen regulation of insulin secretion and signaling in the non-pregnant baboon

This form applies to IACUC protocols using materials such as radioisotopes, infectious agents, carcinogens, biohazards, mutagens, teratogens, abortifacents, acutely toxic chemicals, or other potentially hazardous chemicals. Information provided in this form will enable assessment of risk to individuals handling or caring for animals, as well as risk to other animals. **If you need assistance answering any of the questions, please contact the Environmental Health and Safety Office at (757[redacted].**

The Division of Comparative Medicine (CompMed) must be notified in writing at least one week prior to the use of hazardous agents in animals. The use of hazardous agents in animals is not permitted until approval has been granted by the CompMed Facility Manager. Please reference the CompMed SOP entitled, *Appropriate Notification, Signage and Protocol for Working with Hazardous Agents in Animal Holding Rooms and Procedure Rooms.*

YOU MUST COMPLETE A SEPARATE FORM FOR EACH HAZARDOUS AGENT USED.

1. Please indicate the type of hazardous agent being used. Check all that apply. For microorganisms, please list the Risk Group or Biosafety Level. For chemicals, please list the CAS # and the LD50 from the Material Safety Data Sheet (MSDS).

    ____ Radioactive material (Isotope: _____)
    ____ Infectious agent (Risk Group or Biosafety Level: _____)
    ____ Acutely toxic chemical (CAS #: _____, LD50: _____)
    ____ Known or suspected human carcinogen (Chemical Name: _____)
    __X__ Known or suspected human mutagen/teratogen (Chemical Name: Letrozole)
    ____ Recombinant DNA/RNA
    ____ Tissue sample (Type: _____)
    ____ Other (*Describe below*):

2. Please provide specific information about the agent:

Complete name
(Include strain for microorganisms): Letrozole

Dose and frequency of administration: 0.2 ml/day for 5 days

Concentration: 35 µg/kg/BW/day

Route: SC Duration of exposure: 5 days

How long will the animal be maintained after administration? Up to 3 years

Animal species: Papio anubis Estimated animal weight: 14-17kg

3. Is the agent excreted or shed by the animal?

_____ YES *(Indicate the type of excreta and estimated quantity per day)*

X NO

4. Are there documented human risks from exposure to the agent? (Risks may be determined from the MSDS or from references found in the Biosafety Manual.)

X YES *(Complete Questions 4a-4e)* _____ NO *(Skip to Question 5)*

4a. Indicate the route(s) of human exposure:

X Inhalation
X Ingestion
_____ Other (describe below):
X Contact
_____ Parenteral

4b. Describe the risks associated with the agent (mutagen, teratogen, etc.):

Letrozole is provided as a gift to our laboratory by the Novartis Corporation and is pharmaceutical grade. Rather than receiving this as a "pill" which have other components and a predetermined dose we obtain the same drug in its original form as a powder which we can then weigh out and dissolve in oil suitable for injection sc at varying doses. A certificate of analysis of the compound is provided to us and a copy can be provided to the IACUC if necessary.

Letrozole is known to suppress estrogen production in females; Letrozole is an oral, anti-estrogen drug that is used for treating postmenopausal women with breast cancer. We will be administering 0.035 mg/kg or approximately 0.5 mg/day per baboon/animal of this agent and which is dissolved in sesame oil. Thus, risk of exposure to humans is very low. However, if exposed, the risk or problem would be the same as that from an acute withdrawal of estrogen; the drug needs to be taken repeatedly to continue to suppress estrogen production; in other words, one exposure will reduce estrogen for one or two days maximally; i.e. the drug is metabolized.

4c. Indicate the acutely toxic/infectious dose in humans (in mg/kg or number of organisms):

The standard dose of Letrozole is 2.5 mg. We will be administering 0.035 mg/kg or approximately 0.5 mg/day per animal of this agent dissolved in sesame oil. The latter is 20% of the standard dose used in women. Thus, at this low level and short duration we do not anticipate baboons will experience any acute side-effects.

Only the PI and staff are exposed to the agent

4d. Describe any genetic changes to the organism and their suspected effects: None expected

N/A

4e. Describe the symptoms of exposure: Exposure would have to be long term and contact would require that the drug be ingested or there be contact with an open wound. Basic exposure symptoms would likely include irritation or skin rash.

4f. Describe the first aid methods to be taken in the case of exposure:

Wash thoroughly with soap and water and flush the area with water

4g. Indicate all personal protection required:

X	Lab coat/dedicated clothing	____	Apron
X	Gloves	X	Face shield
X	Goggles	____	Respirator
X	Other (describe below): Mask		

5. Are there risks to other animals in the room or in the animal facility?

____ YES *(Complete Questions 5a-5d)* X NO *(Skip to Question 6)*

5a. Describe the risk to other animals:

5b. Indicate the route of animal exposure:

5c. describe all methods that will be used to contain the risk factor:

5d. Are special animal care requirements necessary?

____ YES *(Describe below)* ____ NO

6. Are special waste or carcass disposal requirements necessary?

____ YES *(Describe below)* X NO

7. Briefly describe the procedure(s) for administration of the agent and how the agent is metabolized and/or excreted. Include how long the agent is expected to remain in/be shed from the animal, the concentration of agent in any specific organs, etc.

7a. Please indicate whether or not the laboratory personnel and CompMed staff have been informed of these hazards and have received training on proper handling techniques.

Laboratory Personnel		**CompMed Staff**	
X	Yes	X	Yes
____	No	____	No

If not, please contact the Environmental Health and Safety Office to schedule the proper training.

8. Please provide any other relevant information regarding the agent (e.g., unique containment recommendations):

This original agent is in powder form and prepared by Dr. Pepe and or his staff and dissolved in sesame oil and injected SC by PI staff.

Eastern Virginia Medical School
Institutional Animal Care and Use Committee
Attachment E: Animal Surgery

Project Title: Estrogen regulation of insulin secretion and signaling in the non-pregnant baboon

A. PRE-OPERATIVE PROCEDURE

All animal activity proposals involving surgery must provide specific details of pre- through post-procedural care and relief of pain and distress.

1. List by name the person(s) who will be responsible for evaluating the health status of the animals?

   Dr. Pepe, [redacted], and CompMed staff

2. Will food be withheld?

   X YES *(Please explain below and indicate how long food will be withheld)* ____ NO

   Food is withheld prior to sedation and anesthetization for blood draw, and/or glucose tolerance test, and surgical procedures, generally 12-18 hours prior to sedation.

3. List all pre-operative anesthetics/analgesics to be used:

   Ketamine: IM
   Xylazine: IM
   Isoflurane: Inhalant
   Flunixin meglumine, IM

4. Describe briefly how animals will be prepared for surgery:

   Animals will be prepped for surgery according to CompMed surgical SOP. PI staff is available for assistance if needed. An IV catheter is placed in an antecubital or saphenous vein to administer fluids (Saline fluid) and for blood sampling. The animal is draped using sterile technique.

B. ANESTHETIC PROCEDURE

5. Will animals be anesthetized?

   X YES *(Complete Questions 6-8)* ____ NO *(Explain below, then skip to Section C)*

6. Who will administer the anesthesia?

   Comp Med will administer anesthesia; if unavailable, PI trained staff can be employed.

7. What anesthetic will be used (name and dosage) and how will it be administered? Who will keep the records?

   Animals will be intubated and isoflurane / O_2 administered to maintain sedation. All medical records are maintained in the Department of CompMed.

8. Explain how anesthetic recovery will be monitored and indicate the person(s) responsible for monitoring the recovery.

   Immediate post operative monitoring is performed by CompMed and follows CompMed SOP.

C. POST-OPERATIVE PROCEDURE:

Investigators should refer to the EVMS Post-Operative Care Guidelines when developing post-operative procedures.

"Appropriate post-operative records must be maintained in accordance with professionally accepted veterinary procedures regardless of the location of the animal. The attending veterinarian retains the authority to change post-operative care as necessary to ensure the comfort of the animal." AWA, Section 13 and 9 CFR, April 14, 1997.

9. Who will monitor post-operative care on a daily basis?

PI staff will monitor and record recovery in the animal record sheets. CompMed will be employed if PI staff is unavailable.

10. Who will keep the post-operative record and where will the records be maintained?

CompMed will keep the record in the animal record sheets in the CompMed office.

11. Will post-operative analgesics be administered?

X YES _____ NO *(Explain below, then skip to Section D)*:

12. Provide the following information about post-operative analgesia administration:

Agent: Flunixin meglumine

Dose and Route: 2mg/kg IM Frequency: At surgery (IM), then 4-8 hours later, BID on days 2 and 3

Post-Operative Duration of Care: Monitoring for 7 days

D. MULTIPLE SURVIVAL SURGERY

All multiple survival surgery must be conducted in accordance with EVMS' Multiple Survival Surgery Policy.

The Animal Welfare Act does not allow for animals to undergo more than one (1) survival surgery unless scientifically justified by the principal investigator, appropriate for the animal's veterinary care, or approved by the USDA administrator. The IACUC must specifically review and approve every additional surgery to be performed on a single animal.

13. Will the animal be subjected to more than one survival surgery?

X YES *(Answer Questions 13a-13b)* _____ NO *(Skip to Question 14)*

13a. Please briefly outline the procedures, explain how the surgeries are related and justify the need for more than one surgery per animal:

In these experiments, animals will undergo two survival surgeries. Thus, animals are bilaterally ovariectomized and undergo tissue biopsy (i.e. visceral adipose, skeletal muscle and pancreas) during laparoscopic/laparotomy surgery (surgery one) and approximately 8-10 weeks later a second laparoscopic/laparotomy (surgery two) will be performed for purpose of tissue biopsy (adipose, pancreas and skeletal muscle). The final/terminal surgery laparotomy will be performed for sequential tissue biopsy (visceral adipose, pancreas and skeletal muscle) after acute iv administration of estradiol or glucose or estradiol and glucose together and animal then euthanized. To reduce the number of animals required to obtain statistically significant and scientifically valid results, a longitudinal design will be employed in which each animal will pass through each of the different phases/treatment regiments

13b. How many surgeries will each animal experience?

2 survival, 1 terminal

14. Have any animals to undergo major survival surgery in this protocol already undergone major survival surgery in any other IACUC protocols?

_____ YES *(Answer Questions 14a-14d)* X NO *(Skip to Section E)*

14a Identify all animals that have undergone prior surgical procedures in another protocol:

14b. Identify all of the previous procedure(s) involved:

14c. Identify the IACUC protocol number(s) the previous surgeries were performed under:

14d. In accordance with the Animal Welfare Act, the IACUC must specifically review and approve all requests for the use of animals that have undergone previous survival surgery under another IACUC protocol. Please justify the need to reutilize such animals in this surgical protocol:

E. SURGICAL PROCEDURES:

YOU WILL NEED TO SUBMIT A SEPARATE COPY OF THIS PAGE FOR EACH SURGICAL PROCEDURE TO BE PEFORMED.

YOU MAY MAKE ADDITIONAL PHOTOCOPIES OF THIS PAGE AS NEEDED.

15. Is the surgical procedure to be addressed on this page a major surgical procedure or a minor surgical procedure (a major surgical procedure is defined as involving penetration of a body cavity and/or resulting in a disability or dysfunction in the animal):

X Major surgical procedure _____ Minor surgical procedure

16. Is this survival surgery?

X YES _____ NO

17. Indicate what number the surgery described on this page is (i.e., only procedure to be performed, 1st surgical procedure, 2nd surgical procedure, etc.) If you are performing the same exact surgical procedure more than once on a single animal, indicate below how many times you will be performing this procedure on the animal (in this case, you will only need to submit one copy of this page for that specific procedure):

1st survival surgical procedure

18. Indicate the location (provide a specific room number if surgery will be conducted outside of the CompMed animal facility, what time of day and what day(s) of the week surgery will be performed. Take into consideration the time necessary for care after anesthesia and surgery:

Surgery will be performed in the animal facility on the [redacted] floor of [redacted] Monday – Friday

Describe the entire surgical procedure:

The goal of these studies is to test the hypothesis that the ovarian hormone estrogen regulates insulin secretion from the pancreas and insulin action/signaling within target tissues (i.e. skeletal muscle, visceral adipose).

Baboons will be prepped for surgery following CompMed surgical prep SOP. A catheter is placed in antecubital and/or saphenous vein for fluid administration, blood sampling and drug administration. A midline and 1-2 lateral abdominal incisions are made and insufflation of the abdominal cavity with CO_2 gas is initiated. A trocar is used to establish 2-3 ports into the abdomen for laparoscopic camera insertion and 1-2 ports for surgical manipulation of internal organs and a biopsy (experimental time 0 min) of visceral and subcutaneous adipose (fat) will be obtained. The pancreas will be identified and isolated with a retractor (laparotomy only) and a 2mm Bergstrom Stiles needle (Cadence Science, Lake Success, NY) inserted from the tail region through the center of the gland and the needle withdrawn to obtain a biopsy (thread). A biopsy of the quadriceps muscle will be obtained by making a small incision via a #10 surgical blade in the skin and fascia to expose the underlying muscle and using a 8 mm sterile punch biopsy. Hemostasis will be attained by direct pressure applied to the biopsy sites and sealing with Gel-foam. If the laparoscopic procedure poses problems, e.g. biopsy not attainable or bleeding not easily controlled, biopsies and/or subsequent ovariectomy will be performed by laparotomy. Briefly, an incision using a #10 surgical blade will be made to expose both the pancreas and reproductive tract (i.e. laparotomy) and a biopsy of visceral and subcutaneous adipose tissue obtained.. The pancreas will be isolated with a retractor and/or lap sponge and a biopsy obtained using a 2 mm Bergstrom Stiles needle as described above. A biopsy of the quadriceps muscle will be obtained using a 8 mm sterile punch biopsy after making a small incision (#10 surgical blade) in the skin/fascia to expose the muscle. Hemostasis will be attained by direct pressure and application of Gel-foam. Following collection of biopsy specimens, a bolus injection of dextrose (0.25 g/kg BW) into an antecubital vein will then be made and 20 min later biopsies of visceral adipose, pancreas, and quadriceps muscle again obtained at sites at least 2 cm removed from the first biopsy. Animals are then be bilaterally ovariectomized either using laparoscopic procedure or following laparotomy. Briefly, the ovaries are localized and a retractor and lap sponge used to clear the field(laparotomy). The ligaments and associated blood vessels of the right and left ovaries will be clamped and cauterized and both ovaries excised. The clamps will then be carefully removed, the site observed for absence of bleeding and the abdominal cavity rinsed with sterile saline (laparotomy). Incisions will be closed using absorbable suture and vet-bond skin adhesive applied. Isoflurane will then be shut off and the animal monitored until a swallowing reflex is present. The animal will then be extubated and continued to be monitored in home cage until fully upright and responsive to stimuli. The animal is treated with analgesics, vital signs and blood chemistries determined to ensure physiologic homeostasis. The animal is recovered following CompMed immediate post op monitoring SOP. Post-op is monitored for seven (7) days by PI staff.

E. SURGICAL PROCEDURES:

YOU WILL NEED TO SUBMIT A SEPARATE COPY OF THIS PAGE FOR EACH SURGICAL PROCEDURE TO BE PEFORMED.

YOU MAY MAKE ADDITIONAL PHOTOCOPIES OF THIS PAGE AS NEEDED.

15. Is the surgical procedure to be addressed on this page a major surgical procedure or a minor surgical procedure (a major surgical procedure is defined as involving penetration of a body cavity and/or resulting in a disability or dysfunction in the animal):

_X___ Major surgical procedure _____ Minor surgical procedure

16. Is this survival surgery?

_X___ YES _____ NO

17. Indicate what number the surgery described on this page is (i.e., only procedure to be performed, 1st surgical procedure, 2nd surgical procedure, etc.) If you are performing the same exact surgical procedure more than once on a single animal, indicate below how many times you will be performing this procedure on the animal (in this case, you will only need to submit one copy of this page for that specific procedure):

2nd surgical procedure -

18. Indicate the location (provide a specific room number if surgery will be conducted outside of the CompMed animal facility, what time of day and what day(s) of the week surgery will be performed. Take into consideration the time necessary for care after anesthesia and surgery:

Surgery will be performed in the animal facility on the [redacted] of [redacted], Monday – Friday

19. Describe the entire surgical procedure:

Ovariectomized female baboons treated with estradiol will be prepped for surgery following CompMed surgical prep SOP. Baboons will be prepped for surgery following CompMed surgical prep SOP. A catheter is placed in antecubital and/or saphenous vein for fluid administration, blood sampling and drug administration. A midline and 1-2 lateral abdominal incisions are made and insufflation of the abdominal cavity with CO_2 gas is initiated. A trocar is used to establish 2-3 ports into the abdomen for laparoscopic camera insertion and 1-2 ports for surgical manipulation of internal organs and a biopsy (experimental time 0 min) of visceral and subcutaneous adipose (fat) will be obtained. The pancreas will be identified and isolated with a retractor(laparotomy only) and a 2mm Bergstrom Stiles needle (Cadence Science, Lake Success, NY) inserted from the tail region through the center of the gland and the needle withdrawn to obtain a biopsy (thread). A biopsy of the quadriceps muscle will be obtained by making a small incision via a #10 surgical blade in the skin and fascia to expose the underlying muscle and using a 8 mm sterile punch biopsy. Hemostasis will be attained by direct pressure applied to the biopsy sites and sealing with Gel-foam. If the laparoscopic procedure poses problems, e.g. biopsy not attainable or bleeding not easily controlled, biopsies will be obtained by a laparotomy. Briefly, an incision using a #10 surgical blade will be made to expose the pancreas (i.e. laparotomy) and a biopsy of visceral and subcutaneous adipose tissue obtained.. The pancreas will be isolated with a retractor and/or lap sponge and a biopsy obtained using a 2 mm Bergstrom Stiles needle as described above. A biopsy of the quadriceps muscle will be obtained using a 8 mm sterile punch biopsy after making a small incision (#10 surgical blade) in the skin/fascia to expose the muscle. Hemostasis will be attained by direct pressure and application of Gel-foam. Following collection of biopsy specimens, a bolus injection of dextrose (0.25 g/kg BW) into an antecubital vein will then be made and 20 min later biopsies of visceral adipose, pancreas, and quadriceps muscle again obtained at sites at least 2 cm removed from the first biopsy. Incisions will be closed using absorbable suture and vet-bond skin adhesive applied. Isoflurane will then be shut off and the animal monitored until a swallowing reflex is present. The animal will then be extubated and continued to be monitored in home cage until fully upright and responsive to stimuli. The animal is treated with analgesics, vital signs and blood chemistries determined to ensure physiologic homeostasis. The animal is recovered following CompMed immediate post op monitoring SOP. Post-op is monitored for seven (7) days by PI staff.

E. SURGICAL PROCEDURES:

YOU WILL NEED TO SUBMIT A SEPARATE COPY OF THIS PAGE FOR EACH SURGICAL PROCEDURE TO BE PEFORMED.

YOU MAY MAKE ADDITIONAL PHOTOCOPIES OF THIS PAGE AS NEEDED.

15. Is the surgical procedure to be addressed on this page a major surgical procedure or a minor surgical procedure (a major surgical procedure is defined as involving penetration of a body cavity and/or resulting in a disability or dysfunction in the animal):

X Major surgical procedure _____ Minor surgical procedure

16. Is this survival surgery?

_____ YES X NO

17. Indicate what number the surgery described on this page is (i.e., only procedure to be performed, 1st surgical procedure, 2nd surgical procedure, etc.) If you are performing the same exact surgical procedure more than once on a single animal, indicate below how many times you will be performing this procedure on the animal (in this case, you will only need to submit one copy of this page for that specific procedure):

3rd surgical procedure - terminal

18. Indicate the location (provide a specific room number if surgery will be conducted outside of the CompMed animal facility, what time of day and what day(s) of the week surgery will be performed. Take into consideration the time necessary for care after anesthesia and surgery:

Surgery will be performed in the animal facility on the [redacted] of [redacted] Monday – Friday depending on the study parameters

19. Describe the entire surgical procedure:

In the final/terminal surgical procedure, baboons are prepped following CompMed surgical prep SOP. A catheter is placed in the antecubital and/or saphenous vein for fluid administration, blood sampling and drug administration. A midline abdominal incision through skin, fascia/muscle, and peritoneum will be made (laparoscopy will not be utilized for this terminal study). Biopsies of pancreas, visceral adipose and skeletal muscle will be obtained using a 6-mm Bergstrom-Stiles needle at 0, 0.3, 1, 2 and 6 h after acute bolus iv injection of estradiol or glucose or both with hemostasis ensured by direct pressure with sterile gauze and sealing with Gel-foam after each biopsy. Blood samples from an antecubital vein are taken at each of the biopsy time periods. Once biopsies are completed, the animal is euthanized using Pentobarbital IV and sections of tissue then removed from the liver and kidneys.

OFFICE OF RESEARCH | WWW.EVMS.EDU
[redacted] Norfolk, VA 23507 | Phone: 757 [redacted] | Fax: 757 [redacted]

EVMS
Eastern Virginia Medical School

July 30, 2012

Gerald J. Pepe, Ph.D.
Chair, Department of Physiological Sciences
Eastern Virginia Medical School
[redacted]
Norfolk, Virginia 23507

Dear Dr. Pepe: Jerry

Your response to the July 14, 2012 letter regarding the protocol entitled, ***Regulation of Fetal-placental Development in the Primate*** **(IACUC #12-010)**, has been reviewed and accepted by the Institutional Animal Care and Use Committee. **The project is now approved for one year.** Continued approval beyond this point will require submission of an annual progress report, no later than **June 10, 2013**.

Although the Division of Comparative Medicine (CompMed) will make every effort to provide housing, husbandry, and veterinary support for your research project, IACUC approval of this protocol does not guarantee that CompMed will be able to accommodate the work immediately. Available space and resources within the CompMed animal facility are determined by the number of ongoing projects and the animal census at any given time. Please consult with the CompMed management team to confirm the project start date.

It is the responsibility of the principal investigator to notify the Committee, in writing, of any deviation from the IACUC-approved protocol. Please note that a change in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian prior to submitting the amendment request to the IACUC for approval.

It is also the responsibility of the principal investigator to provide for regular observation of the health of the research animals and to provide CompMed with the name(s) and telephone number(s) of the person(s) who may be contacted and/or take action in the event that the CompMed staff notes an emergency condition with the research animals.

Thank you for your continued cooperation with the Institutional Animal Care and Use Committee.

Sincerely,

[redacted]
[redacted]
Institutional Animal Care and Use Committee

[redacted]

cc: [redacted]
Attending Veterinarian
Division of Comparative Medicine

[redacted]
Program Manager
Division of Comparative Medicine

[redacted]
Associate Dean for Research
Institutional Official

EASTERN VIRGINIA MEDICAL SCHOOL
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

FOR IACUC USE ONLY:	
IACUC Number 12-010	Review Date(s)
NOTES: Revision #2/FINAL	Final Approval Date 7/20/12
	Progress Report Due 6/10/13, 6/10/14

Submission Instructions: Submit the original signed typed form to the IACUC Office located in [redacted], and e-mail the MSWord version of the form to the IACUC Administrator no later than 5:00 p.m. on the submission deadline date. **Forms received after the submission deadline will be held for review at the next IACUC meeting.** For assistance, please contact the IACUC Administrator at [redacted].

Initial Review Form for New Animal Care and Use Protocols

PROJECT TITLE. *(If the project title is different from the grant title, please list both titles below.)*
Regulation of Fetal-Placental Development in the Primate

Is this a 3-year renewal of an existing IACUC protocol?	☐ NO	☒ YES	Related IACUC #	09-007

SPECIES INFORMATION: (*In addition to the species, please list the strain(s), if applicable, the sex(es.) and the age(s) of the animals.*)
Baboon (Papio anubis/cynocephalus). Adult female (7-15 years old) and male (8-20 years old) baboons as well as offspring born to animals in the colony and studied in the pre-and post pubertal period and as adults.

Principal Investigator:	Gerald J. Pepe, Ph.D.		
Mailing Address:	Department of Physiological Sciences [redacted]		
Phone: Office	[redacted]	Home:	[redacted]
Lab:	[redacted]	E-mail:	[redacted]@evms.edu

Animal Emergency Contact Person:	[redacted]		
Phone: Office	[redacted]	Home:	[redacted]
Lab:		E-mail:	[redacted]@evms.edu

Technical Coordinator:			
Phone: Office		Home:	
Lab:		E-mail:	

Co-Investigator #1:			
Phone: Office		Home:	
Lab:		E-mail:	

Co-Investigator #2:			
Phone: Office		Home:	
Lab		E-mail:	

Co-Investigator #3:			
Phone: Office		Home:	
Lab		E-mail:	

Co-Investigator #4:			
Phone: Office		Home:	
Lab		E-mail:	

LIST ALL PROJECT SITES:					LIST THE PROJECT PERIOD		
Bldg:	[redacted]	Room(s):	[redacted]		From: 7/12/12		To: 7/11/15
Bldg:	[redacted]	Room(s):	[redacted]				

FUNDING SOURCE(S):	***Please check all that apply.*** ☐ Federal Government ☐ State or Other Government ***Specify Source:*** _____ ☐ Private ☐ Industry ☒ Campus/Department Funds ☒ Other ***Specify source:*** Start up		
STATUS OF FUNDING:	☐ Approved	☐ Pending	☒ Not Applicable
Is a committee approval verification letter needed for the funding source(s)?	☒ NO Please note that it is the investigator's responsibility to inform the funding agency of any changes to the animal protocol. Any changes must also be approved by the IACUC before they are implemented.	☐ YES (***Complete Attachment A, REQUEST FOR A LETTER OF VERIFICATION***) ☐ Final copy of grant attached Please include a final copy of the grant to permit comparison of the animal work described in the grant with the animal work described in the *Initial Review Form.*	

OTHER COMMITTEE REVIEWS:

Prior to initiation of this project, approval must be acquired from the appropriate committees or offices.

Please complete the following table as it pertains to your protocol. If applicable, complete ***Attachment D, USE OF HAZARDOUS AGENTS.***

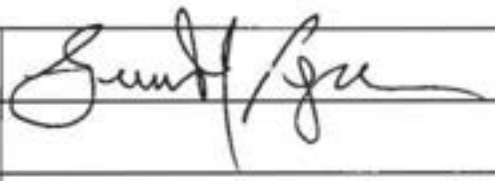
Project Involves:	Yes	No	Committee/Office	Certification Number	Hazard to:	
					Personnel	Animals
Radioisotopes, *in vivo*		X	EVMS Radiation Safety Committee *(Complete **Attachment D**)*			
Recombinant DNA, RNA, All Tissue or Cell Samples, Laboratory-Induced Infection, or Cultured Pathogens	X		EVMS Institutional Biosafety Committee *(Complete **Attachment D**)*	7/13/09	X	
Known or Suspected Chemical Hazards, Mutagens or Teratogens	X		EVMS Office of Environmental Health & Safety/Radiation Safety *(Complete **Attachment D**)*		X	
Lasers or Penetrating Electromagnetic Radiation with Living Animals		X	EVMS Office of Environmental Health & Safety/Radiation Safety			
Other (*Please describe*):		X				

PRINCIPAL INVESTIGATOR'S ASSURANCES:

I hereby certify that:

- no animal involved in this project will be subjected to discomfort, pain, or distress, unless it is unavoidable in the conduct of scientifically valuable research;
- any such discomfort, pain, or distress will be alleviated with the appropriate anesthetic, analgesic, or tranquilizing drugs, unless specific approval for not using these agents is given by the Committee;
- the project will be carried out within the provisions of the Animal Welfare Act (Public Law 99-198), the National Research Council (NRC), the Public Health Service Policy on Humane Care and Use of Laboratory Animals (PHS), the "Guide for the Care and Use of Laboratory Animals (1996)", the Health Research Extension Act of 1985 Public Law 99-158 (11/20/86), and United States Department of Agriculture (USDA) regulations;
- all procedural and/or personnel changes will be brought to the attention of the IACUC through the amendment process, prior to implementation, understanding that failure to request an amendment for changes in animal use may place me and the Institution in violation of federal regulations and the Animal Welfare Act;
- the details of the research to be conducted in this protocol are consistent with the details of the research as written in any grant, contract, or subcontract related to or connected with this protocol;
- all personnel using animals have completed the appropriate training requirements to assure the humane, safe, and appropriate use of animals in this context.

The signatures below signify assurance that the individuals involved will comply with the project as described herein.

Principal Investigator:		Date:	June 27, 2012
Technical Coordinator:		Date:	
Co-Investigator #1:		Date:	
Co-Investigator #2:		Date:	
Co-Investigator #3:		Date:	
Co-Investigator #4:		Date:	

DEPARTMENT CHAIR'S ENDORSEMENT:
I have reviewed the research project which is the basis for this submission. I am aware of and approve of the procedures outlined for this study. I agree to take ultimate fiscal responsibility for the payment of animal care charges.

Department Chair or Designee:	[redacted]	Date:	6/27/12
Printed or Typed Name:			

VETERINARY CONSULTATION:
The IACUC requires a **MANDATORY** consultation with the veterinarian to provide the investigator with information that is relevant to the species and study procedures. It is the responsibility of the investigator to incorporate the appropriate information into the protocol **BEFORE** it is submitted to the IACUC for review. The veterinarian's signature below **does not** constitute an approval of the protocol. The signature merely acknowledges that a consultation with the veterinarian has occurred.

EVMS Veterinarian		Date:	
Printed or Typed Name:	[redacted]		

IACUC APPROVAL:

IACUC Chair or Designee:		Date:	
Printed or Typed Name:			

DEPARTMENT CHAIR'S ENDORSEMENT:
I have reviewed the research project which is the basis for this submission. I am aware of and approve of the procedures outlined for this study. I agree to take ultimate fiscal responsibility for the payment of animal care charges.

Department Chair or Designee	[redacted]	Date	5/24/12
Printed or Typed Name			

VETERINARY CONSULTATION:
The IACUC requires a **MANDATORY** consultation with the veterinarian to provide the investigator with information that is relevant to the species and study procedures. It is the responsibility of the investigator to incorporate the appropriate information into the protocol **BEFORE** it is submitted to the IACUC for review. The veterinarian's signature below **does not** constitute an approval of the protocol. The signature merely acknowledges that a consultation with the veterinarian has occurred.

EVMS Veterinarian	[redacted]	Date	5-23-2012
Printed or Typed Name			

IACUC APPROVAL:

IACUC Chair or Designee	[redacted]	Date	8/1/12
Printed or Typed Name			

A. PROTOCOL OBJECTIVE:

In clear, concise, non-technical, lay language *(i e. the type of writing style used in newspapers)*, summarize the background, general hypothesis, experimental plan, and relevance of the study to the advancement of scientific knowledge and/or the benefits to human and animal health. All abbreviations must be defined. **Scientific abstracts from grant applications or journal articles are not acceptable.**

Goal: It is well known that the hormone estradiol, also called estrogen and produced by a women's ovary, acts on several tissues and elicits effects beneficial to women's health. Thus, cessation of estrogen after menopause increases risk for development of cardiovascular disease, diabetes, and bone loss as just a few examples. Studies, including those in our laboratories using the baboon as a model for the human, have now confirmed that estrogen also plays a critical role in pregnancy and is required for pregnancy to proceed, for the fetus/baby to grow and be delivered and ultimately develop outside the mother's womb. Uniquely in humans and nonhuman primates such as the baboon but not animals such as rodents, while the mother's ovary remains the source of estrogen during the first trimester, the placenta becomes the producer of estrogen thereafter. Moreover, placental estrogen production requires participation of the fetus. Thus the fetus, mother and placenta interact and actually communicate with each other via the hormone estradiol. Unfortunately, it is impossible to perform in women invasive experiments that interrupt this maternal-fetal-placental communication to study the role of estrogen. Therefore, our understanding of the sites and means by which estrogen works and assures that the fetus grows remains incomplete. As a consequence, the incidence of premature birth, poor fetal growth, maternal diseases such as high-blood pressure/pre-eclampsia remain high. Also, unlike many years ago, women today are exposed to compounds in the environment known as endocrine disruptors several of which e.g. bisphenol A (BPA) either enhance or inhibit the actions of estradiol.

Using the baboon as a translational research model for the human, we recently showed that exposure of the mother early in pregnancy to a very small increase in estradiol significantly decreased remodeling of mom's uterine blood vessels by placental cells. This process which is essential for development of normal maternal and fetal cardiovascular function in pregnancy is also apparently decreased in women with preeclampsia. The present proposal outlines studies designed to determine the sites and mechanisms of estrogen action and how fetal blood flow becomes reduced/compromised. We also propose studies to determine whether the negative impact on fetal blood flow is still apparent when the fetus is born and develops as an adult. By knowing how and sites/factors regulated by estrogen, we can design studies in women to begin to determine who might be at risk and design methods/approaches to reduce the impact of the disease.

Our laboratories also showed that inhibition of the increase in placental estradiol production in the second half of pregnancy altered development of key organs in the fetus. In addition, babies born to mothers in which placental estradiol was suppressed exhibited a reduced response to insulin, a condition known as insulin resistance. We also showed that the development of the ovary in female fetuses and the testes in male fetuses was impaired. Moreover, in offspring of estrogen-suppressed mothers that we reared to adulthood, reproductive function was compromised. Thus, estrogen-suppressed animals exhibited a delay in onset of puberty and a pattern of estrogen production during their menstrual cycles that was typical of that in women with early (premature) menopause. The sites and mechanisms by which estrogen is working is the goal of the second series of experiments outlined in this protocol.

To accomplish our goals and study the role of estrogen in pregnancy, pregnant baboons will be treated with estradiol or a specific inhibitor of estrogen synthesis alone or in combination with estrogen. Treated/untreated animals will be delivered by cesarean section at early, mid or late gestation and the placenta and fetal tissues collected and studied for aspects of biochemical/physiologic maturation. In other experiments, treated/untreated animals will be delivered near term and neonates reared to adulthood. Development of vascular function (e.g. ability to control blood pressure), blood vessel flow and glucose regulation as indexes and/or predictions of development of diabetes and adrenal and ovarian function will also be determined. These studies serve as a model for the human and are designed to provide new information which will enhance our understanding of the causes of pregnancy complications in women (e.g. preeclampsia; fetal growth retardation and prematurity *per se*) and the role of hormones *in utero* on programming fetal organ systems critical for development of appropriate vascular (e.g. blood pressure), metabolic (e.g. glucose-diabetes) and reproductive function in adulthood.

B. SEARCH FOR ALTERNATIVES

In an effort to minimize pain and distress, the Animal Welfare Act (AWA) regulations require principal investigators to consider alternatives to procedures that may cause more than momentary or slight pain or distress to animals. The AWA also requires principal investigators to provide a written narrative of the methods used and sources consulted to determine the availability of alternatives, including replacements, reductions, and refinements of animal use. These alternatives should be consistent with the goals of the proposed research. *Potential alternatives that do not allow the attainment of the goals of the proposed research are not, by definition, alternatives.* The "3 Rs" are defined below:

REPLACEMENT: *An alternative that will be equally informative. Replacements include, but are not limited to, in vitro models, in silico methods, invertebrate models, and vertebrate models.*

REDUCTION: *Reducing the number of animals to the minimum required to obtain scientifically valid data and demonstrating that the proposed research does not unnecessarily duplicate previous work.* Reduction includes statistical methods to reduce animal numbers, and it addresses whether or not animals can be reused for other purposes.

REFINEMENT: *A procedure that lessens or eliminates pain or distress, thereby enhancing animal well-being.* Housing, environmental enrichment, animal identification, anesthesia, analgesia, and euthanasia procedures can be refined, in addition to activities normally thought of as procedures, such as surgeries, tissue or fluid collection, etc.

The fundamental goal of the AWA and USDA Policy 12 (June 21, 2000) is to minimize pain and distress to animals; consequently, the regulations state that any proposed animal activity or significant changes to an ongoing animal activity must include the following: (1) a rationale for involving animals, and the appropriateness of the species and the number of animals to be used; (2) a description of the procedures or methods designed to assure that discomfort and pain to animals will be limited to that which is unavoidable in the conduct of scientifically valuable research, and that analgesic, anesthetic, and tranquilizing drugs will be used where indicated and appropriate to minimize discomfort and pain to animals; (3) a written narrative description of the methods and sources used to consider alternatives to procedures that may cause more than momentary or slight pain or distress to the animals, and; (4) the written assurance that the activities do not unnecessarily duplicate previous experiments.

DATABASE SEARCHES

A database search is considered to be the most effective and efficient method for demonstrating compliance with the federal regulations for consideration of alternatives to painful and distressful procedures, although other sources, such as conferences, colloquia, subject expert consultation, etc., may provide relevant and up-to-date information regarding alternatives, in lieu of or in addition to a database search. **Institutional policy requires investigators to specify at least two (2) databases or other acceptable sources** that were used to determine that alternatives to animals have been considered, that the minimal number of animals have been requested, that the proposed research is not duplicative of previous work, and that alternatives to procedures that may cause more than momentary or slight pain or distress to the animals have been considered. For all database searches, the following information **must** be provided: (1) the name of the database; (2) the date the search was performed; (3) the time period covered by the search, and; (4) the key words and/or the search strategy used.

Please be sure to list all key words and key word combinations used and the number of citations found for each key word or combination [*e.g. amiloride mouse kidney (455 citations), mouse hemizona assay (453 citations)*]. **PLEASE NOTE: The search must include the key word "pain" and any relevant combination thereof.** Be sure to search for all applicable terms, including the search for alternatives [*e.g. mouse heart computer model (55 citations)*]. Use the widest possible time range to include both modern and classical references.

EXPERT CONSULTATIONS

An appropriate, well documented consultation with an expert in the field of the proposed research can replace a second database search. In order to demonstrate to the IACUC the expert's knowledge of the availability of alternatives in the specific field of study, documentation of the consultation **must** include the following: (1) the consultant's name and qualifications; and (2) the date and content of the consultation.

DESCRIPTION AND JUSTIFICATION

Regardless of the sources used to search for alternatives, the written narrative should include adequate information for the IACUC to assess that a reasonable and good faith effort was made to determine the availability of alternatives to animals or procedures. If the database search or another acceptable source identifies an alternative that could be used to accomplish the goals of the proposed research; however, the investigator chooses not to use that alternative, the investigator must provide a written narrative justifying why the alternative was not used.

1. **Database and Literature Search:**

	Yes (X)	Date Search Conducted	Key Words/Search Strategy	Time Period Covered by Search
Databases/Computer Systems				
AGRICOLA Database (National Agriculture Libr.) Searcher – [redacted]	X	6/6/06 5/5/09 **5/22/12**	Estrogens/estrogen receptor modulators, etc (3029); embryonic/fetal growth/development (2301); gonad, testis, adrenal glands, adipose tissue, skeletal muscle, placenta, pancreas, etc (644); menstrual cycle, pre-eclampsia, insulin resistance, fertility, angiogenesis, etc.(11137); cesarean section (356); papio/baboons (184); animal use alternatives, animal welfare, animal models, pain/stress/distress/ suffering, refine/replace/ reduce, humane endpoint, etc. (44354). **Citations = 2, 0**	1970-2006 2006-2009 **updated 2009-2012**
MEDLINE Database Searcher – [redacted]	X	6/6/06 5/5/09 **5/22/12**	Estrogens/estrogen receptor modulators, etc (22065); embryonic/fetal growth/development (37901); gonad, testis, adrenal glands, adipose tissue, skeletal muscle, placenta, pancreas, etc (33487); menstrual cycle, pre-eclampsia, insulin resistance, fertility, angiogenesis, etc.(182052); cesarean section (6749) ; papio/baboons (820); animal use alternatives, animal welfare, animal models, pain/stress/distress/suffering, refine/replace/ reduce, humane endpoint, etc. (1403264). **Citations = 9, 1, 2**	1966-2006 2006-2009 **updated 2009-2012**
CAB Abstracts Database				
TOXLINE				
BIOSIS Database Searcher – [redacted]	X	6/6/06 5/5/09 **5/24/12**	Estrogens/estrogen receptor modulators, etc. (23745); embryonic/fetal growth/development (10412); gonad, testis, adrenal glands, adipose tissue, skeletal muscle, placenta, pancreas, etc. (3095); menstrual cycle, pre-eclampsia, insulin resistance, fertility, angiogenesis, etc. (50216); cesarean section (3117); papio/baboons (1260); animal use alternatives, animal welfare, animal models, pain/stress/distress/suffering, refine/ replace/ reduce, etc. (1101104). **Citations = 12, 6**	1969-2006 2006-2009 **updated 2009-2012**
Other: EMBASE Searcher – [redacted]	X	6/6/06 5/5/09 **5/24/12**	Estrogens/estrogen receptor modulators, etc. (33176); embryonic/fetal growth/development (19251); gonad, testis, adrenal glands, adipose tissue, skeletal muscle, placenta, pancreas, etc. (3654); menstrual cycle, pre-eclampsia, insulin resistance, fertility, angiogenesis, etc. (88396); cesarean section (9922); papio/baboons (832); animal use alternatives, animal welfare, animal models, pain/stress/distress/suffering, refine/replace/reduce, etc. (1462901). **Citations = 16, 7**	1974-2006 2006-2009 **updated 2009-2012**
Literature & Reference Sources				
AAALAS				
Quick Biblio. Series (AGRICOLA)				

Laboratory Animal Welfare Biblio. (NLM)				
Animal Welfare Information Ctr.				

2. List any consultations with investigators in the field (*This consult should be related to replacements, reductions, and/or refinements and not simply to the science behind the research.*)
N/A

3. Please provide a brief narrative regarding search methods utilized, but not listed above:
The research outlined in this protocol has consistently developed and been supported in large part by NIH R01 HD 13294 and NIH U54 HD 36207. Both grants have been peer reviewed and HD 13294 funded by NIH continuously since 1980 and funding approved for an additional 5 years in 2007. The U54 grant was funded for the period 1997-2010. Although the latter is no longer funded by NIH, aspects of work/studies developed under the auspices of this grant are still in progress and funded by departmental sources and data being published and to be used as supportive rationale/preliminary data for new grant submissions to the NIH. All previous grant submissions have consistently been viewed as exhibiting outstanding clinical/translational relevance to the human. Since 1981, the research program using the pregnant baboon model to study placental-fetal development has resulted in publication of over 140 manuscripts in peer-reviewed journals with high impact factors (e.g. Endocrinology) as well as seminal review chapters in "Endocrine Reviews". In addition, a search of the literature was performed in consultation with [redacted] librarian at EVMS. The databases searched included: AGRICOLA, Medline, BIOSIS and EMBASE and employed key words most notably pregnancy-fetal development, baboon and baboon-alternatives. The Medline search also employed several other key words (e.g. estradiol, estrogen antagonists, estrogen receptors). The initial search history (1966 through April, 2012) identified (depending on key word) anywhere from 2454 to over 1,013,089 results; a refined search of these hits indicated that none outlined an alternative procedure for the studies we have outlined in our protocol. Moreover, of the several manuscripts cited/abstracts printed as relevant to the search questions, the majority were publications from my laboratory. Although studies using rodents were identified, the rodent (including rat, mouse, guinea pig) is not an acceptable model for studies of human placental-fetal development. Thus, these animals do not have a fetal-placental unit, do not exhibit fetal organ system maturation as occurs *in utero* in the human (e.g. adrenal gland, ovary or testis) and exhibit unique patterns of postnatal development that impact organ system development also not typically noted in the human. Most importantly, type of placenta and thus transfer of maternal substrates across the placenta to the fetus, as well as the fetal hormonal milieu of rodents and even large farm animals (e.g. sheep) are significantly different from that in the human. In contrast, and as substantiated by the literature search, the baboon is a well-established model for studies of human pregnancy.

C. NARRATIVE: *The narrative must address the following:*

4. Provide the rationale/justification for animal use, and discuss what alternatives (e.g. *cell lines, computer simulations or artificial bodies*) were considered, and why the alternatives are not appropriate for this study's objective(s).
There is no way to perform these studies in humans and the potential impact on the development of new treatment regimens for maternal/fetal medicine is profound. For example, clinical trials in Europe show that human females born prematurely and treated neonatally with estradiol and progesterone at levels comparable to what they would have been exposed *in utero* have benefited significantly (e.g. bone maturation, neural development) over their non-treated counterparts. Epidemiologic evidence suggests that inappropriate invasion of the uterine spiral arteries may be the cause of pre-eclampsia but no one has been able to demonstrate cause:effect. Our studies suggest that too much estrogen early in pregnancy may be a causative factor and some women unsuspecting they are pregnant apparently continue to take estrogen-containing birth control pills during the very early stages of pregnancy. Moreover, women in IVF (ART) programs almost always have extremely high levels of estradiol and progesterone in early stages of their pregnancies. Other animals, such as rodents, cannot be used for such studies as they differ (e.g. placentation) considerably from the human. The baboon, like humans has a fetoplacental unit and a similar hormonal profile, placental development and metabolic machinery and fetal adrenal and ovarian anatomy, biochemistry and developmental pattern. Moreover, examination of isolated human tissue or computer simulations does not permit elucidation of the interaction between mother, placenta, and fetus in pregnancy maintenance/fetal development/parturition. Moreover, such studies do not permit testing that what happens *in utero* actually impacts physiological outcome in adulthood. As emphasized in several journals, fetal growth restriction, prematurity, pre-eclampsia and infertility as well as diabetes and hypertension/cardiovascular disease continue to be major health problems in the United States with annual direct costs associated with fetal immaturity alone exceeding that caused by AIDS. In humans, a poorly developed or inadequately functioning placenta results in intrauterine growth retardation/ reduced neonatal birth weight and epidemiologic studies have shown that adults with low birth weight are predisposed to hypertension and reproductive dysfunction. While these clinical studies cannot provide

cause:effect information, they may become more meaningful when interpreted in light of results from our *in vivo* studies in the baboon. Clearly, the experimental baboon model and the multidisciplinary collaborative approach developed by the investigators permit a necessary evaluation of the interactions essential to fetal-placental development. Thus, the results derived from the completion of this study will provide important new insight into the communication that occurs between the fetus and placenta and ultimately improve our knowledge of the regulation of pregnancy maintenance and development of neonatal self-sufficiency and reproductive function in the human. Finally, the search of the databases outlined in Section B question 3, did not identify any alternative methods/procedures for conduct of studies to examine role of pregnancy on placental-fetal development and impact of the hormonal milieu *in utero* on physiologic/reproductive function in adulthood. Rather, the search identified manuscripts that demonstrated the value and acceptability of the baboon as a model for studies of human pregnancy.

5. Discuss the appropriateness of the species (and the animal strain, if applicable) chosen to meet the objective(s) of the study

In the present study, we propose to continue our use of the pregnant baboon as a model to study the developmental regulation of maternal, uteroplacental, and fetal pituitary, adrenocortical/gonadal maturation and function in human pregnancy as well as impact of the intrauterine hormonal milieu on neonatal growth, puberty onset and physiologic (e.g. glucose tolerance; vascular function) and reproductive function (e.g. ovulation; uterine function; fertility; premature menopause) in adulthood. Because the maternal, fetal, and placental units are functionally interrelated during human and nonhuman primate pregnancy, e.g. estrogen biosynthesis, they cannot be evaluated separately. Therefore, *in vitro* approaches utilizing isolated tissues do not on their own permit an assessment of the maternal-fetal-placental endocrine system. As in humans, the baboon possesses a hemochorial and monodiscoid placenta and a functional fetoplacental unit, in which the fetal adrenal gland provides the major portion of C_{19}-steroid precursors required for placental estrogen formation. Because non primate laboratory animals, e.g. the laboratory rat, do not exhibit hemochorial placentation and do not possess a fetoplacental unit for the biosynthesis of hormones such as estrogen and maturation of fetal organ systems including the adrenal and gonad occurs post-natally (i.e. extra-uterine), their applicability to the human is limited.

The qualitative and quantitative hormonal profiles exhibited in pregnant baboons are also similar in many important respects to those in pregnant women. For example, the progesterone production rate and serum progesterone concentrations are elevated during pregnancy in baboons as in women. This contrasts with other nonhuman primates, e.g. rhesus monkeys, in which serum progesterone concentrations and production rates are similar in the pregnant and nonpregnant states. An elevation in the quantities of progesterone in the peripheral circulation is essential to enable their manipulation and thus study of the regulatory factors involved. Similarities in the metabolism of progesterone during baboon and human pregnancy further support the use of the baboon for the study of steroid hormone production. Thus, the major metabolite of progesterone in women and baboons is pregnanediol, while in rhesus monkeys it is androstenedione. The concentrations and patterns of estradiol and estrone in the maternal circulation of pregnant baboons are similar to those in pregnant women, while the concentration of estradiol in rhesus monkeys at term is an order of magnitude less than in women. Corticosteroid production and metabolism also are similar in female baboons and humans. Indeed, the rate of cortisol production and excretion, type and degree of conjugation and formation of tetrahydrocortisol and tetrahydrocortisone as major metabolites are very similar in baboons and women. This contrasts with other non-human primates including most new-world primates (owl, squirrel and marmoset monkeys) in which serum cortisol levels and production rates are excessively high and comparable to those in humans with Cushing Syndrome.

Therefore, the baboon provides an excellent, scientifically valid model for the study of the endocrinology of human pregnancy. Finally, the 30 years of baseline data which this laboratory has obtained in pregnant baboons forms a critically important basis for the continued use of this animal model and further points to the value and peer-reviewed acceptance of the baboon for the study of the endocrinology of human pregnancy. Moreover, the search of the databases outlined in Section B question 3, did not identify any alternative methods/procedures for conduct of studies to examine role of pregnancy on placental-fetal development and impact of the hormonal milieu *in utero* on physiologic/reproductive function in adulthood. Rather, the search identified manuscripts that demonstrated the value and acceptability of the baboon as a model for studies of human pregnancy.

The numbers used are the minimum to permit collection of statistically valid and scientifically meaningful data. Sample size for comparison of means by treatment was determined by estimating the variance as from previous studies (1972-10) in my laboratory and assuming the populations are normally distributed obtained as outlined in Daniel (Biostatistics: A Foundation Analysis in the Health Sciences, 4th Ed., 1987).

6. Describe steps taken to reduce the number of animal in your study. (*e.g. replacement with in vitro procedures, refinement of experimental design, refinement of procedural techniques*)

As indicated above, there is no way to perform these studies in humans and the potential impact on the development of new treatment regimens for maternal/fetal medicine is profound. Other animals, such as rodents, cannot be used as they differ (e.g. placentation) considerably from the human. The baboon, like humans has a fetoplacental unit and a similar hormonal profile, placental metabolic machinery and fetal adrenal

anatomy/biochemistry. Moreover, examination of isolated human tissue or computer simulations does not permit elucidation of the interaction between mother, placenta, and fetus in pregnancy maintenance/fetal development/ parturition. We have refined our experimental designs such that we use the same animal preparation to examine the role of estrogen on placental as well as fetal development and maternal well-being. In other words, we do a single primary experimental manipulation (e.g. injection of estrogen) and monitor the mother throughout the pregnancy (e.g. ultrasound; peripheral blood sampling for hormone and blood chemistries) and examine several aspects of placental (e.g. endovascular invasion; placental microvilli) and fetal organ system (e.g. gonad; adrenal; pituitary) development and function. Finally, the search of the databases outlined in Section B question 3, did not identify any alternative methods/ procedures for conduct of studies to examine role of pregnancy on placental-fetal development and impact of the hormonal milieu *in utero* on physiologic/reproductive function in adulthood. Rather, the search identified manuscripts that demonstrated the value and acceptability of the baboon as a model for studies of human pregnancy.

7. Will the animals be subjected to procedures that may cause more than momentary or slight pain or distress? **NOTE**: These *procedures include environmental, nutritional or behavioral modifications that increase stress, as well as chronic food or water deprivation*

YES **(A database search is required)**

X *Complete* ***Question 8*** _____ NO (*Skip to* ***Question 9***)

8. If alternative procedures have been identified, describe the procedures below, and explain why they are not scientifically appropriate for this research project

The search of the databases outlined in Section B question 3, did not identify any alternative methods/ procedures for conduct of studies to examine role of pregnancy on placental-fetal development and impact of the hormonal milieu *in utero* on physiologic/reproductive function in adulthood. Rather, the search identified manuscripts that demonstrated the value and acceptability of the baboon as a model for studies of human pregnancy.

9. Is the proposed study duplicative of research previously undertaken by the investigator or other scientists? **If yes, describe the duplicative nature of this project and offer scientific justification.**

No. Thus, the search of the databases outlined in Section B question 3, did not identify any alternative methods/procedures for conduct of studies to examine role of pregnancy on placental-fetal development and impact of the hormonal milieu *in utero* on physiologic/ reproductive function in adulthood. Rather, the search identified manuscripts that demonstrated the value and acceptability of the baboon as a model for studies of human pregnancy.

10. Federal regulations require a written rationale/justification for the number of animals to be used. Describe the statistical test (*e.g. power analyses and/or other rationales such as tissue collection needs and breeding efficiency*) used to determine the number of animals required to complete the proposed study, and provide the results of the test. **NOTE: The IACUC may require a consultation with a statistician.**

Throughout the course of conduct of our studies we have consulted a statistician at EVMS (or at the University of Maryland). For example, in studies of fertility, we calculate that with an n of 8 animals per group, there will be approximately 80% power to detect a difference ($P<0.05$, Chi-square with continuity correction) in the proportion of baboon adolescents achieving a pregnancy in the untreated (90%) vs that in estrogen-suppressed (30% to 20%, respectively) baboons. For analysis of the number of samples required to ascertain whether there are statistical differences ($P<0.05$) between populations in tissue morphology and/or expression of mRNA and/or protein biochemical measures (e.g. follicles/0.033 mm^2; estrogen receptor mRNA/unit housekeeping gene) using analysis of variance with post hoc comparison of means by the Neumann-Keuls statistic and appropriate transformation of variables to satisfy ANOVA assumptions, there will be > 80% power to identify differences between the 3 or 4 treatment groups with n=8/group ($\Phi = 2.0$). For analysis of histology and levels of factors in tissue samples from conduct of Studies I and II, statistical differences ($P<0.05$) between populations in tissue morphology (i.e. mean ± SE follicle numbers/0.33 mm^2, placental microvillus number/height) and biochemical development (i.e. GLUT-4 mRNA levels) will be determined by analysis of variance with *post hoc* comparison of the means by the Newman-Keuls statistic and appropriate transformation of variables to satisfy ANOVA assumptions. With n=8/group and pooled estimate of variance (ϕ) of 2.0, there will be at > 82% power to identify differences between the 3 or 4 treatment groups. For the acute glucose tolerance tests and ACTH stimulation and in vitro studies of adrenal function comparison of data at different time points will consist of a repeated measures mixed-model ANOVA with treatment as fixed effect and subject as random effect.

D. USDA PAIN CODE:

11. For each of the appropriate pain code descriptions, list the species (and the animal strain, if applicable) and the number of animals to be used each year. **Please provide the total for all three years.**

Level B				
Breeding or holding colony protocols where animals are not undergoing any manipulation.				
Species	**Year 1**	**Year 2**	**Year 3**	**Total**
Baboon (*Papio anubis/cynocephalus*) adult male breeders	5			5

Level C				
Teaching, research, experiments or tests conducted on animals involving no or momentary/slight pain or distress (i.e., *euthanizing animals for tissues; injections, observation under normal conditions; positive reward projects, use of Acepromazine for vasodilatation in rabbits*), and **for which no pain-relieving drugs are used.**				
Species	**Year 1**	**Year 2**	**Year 3**	**Total**
None				

Level D				
Teaching, research, experiments, surgery, or tests conducted on animals involving a degree of pain or distress (*i.e., non-survival surgery, survival surgery, antibody production; subcutaneous implants, induced infections*) and **for which appropriate anesthetic, analgesic or tranquilizing drugs are used to relieve the pain and distress.**				
Species	**Year 1**	**Year 2**	**Year 3**	**Total**
Baboon (*Papio anubis/cynocephalus*) Female adults and juveniles	28 adult females and 17 juveniles	28 adult females and 17 juveniles	28 adult females and 17 juveniles	84 adult females and 51 juveniles

Level E				
Teaching, research, experiments, surgery or tests conducted on animals involving a degree of pain or distress and **for which the appropriate anesthetic, analgesic or tranquilizing drugs are NOT used because their use will adversely affect the procedures, results, or interpretation of the teaching, research, experiments, surgery or tests. (SCIENTIFICALLY JUSTIFIED IS REQUIRED)**				
Species	**Year 1**	**Year 2**	**Year 3**	**Total**
NONE				

E. STUDY PROCEDURES:

12 Please indicate all procedure(s) that will be performed in the study. **Attach all required forms.**

- [X] Non-Survival Surgery *(Complete **Attachment E, ANIMAL SURGERY**)*
- [X] Single Major Survival Surgery *(Complete **Attachment E, ANIMAL SURGERY**)*
- [X] MULTIPLE Major Survival Surgery *(Complete **Attachment E, ANIMAL SURGERY**)*
- [ ] Prolonged Restraint *(Complete **Attachment C, PROLONGED PHYSICAL RESTRAINT OR STRESS**)*
- [X] Collection of Tissues, Cells or Organs
- [ ] Adverse Conditioning
- [ ] Special Diet
- [ ] Food/Water Deprivation *(Complete **Attachment C, PROLONGED PHYSICAL RESTRAINT OR STRESS**)*
- [X] Use of Biohazards or Chemical Agents *(Complete **Attachment D, USE OF HAZARDOUS AGENTS**)*
- [ ] Burns or Trauma
- [ ] Antibody Production *(Complete **Attachment F, ANTIBODY PRODUCTION**)*
- [ ] X-Rays or other Radiation
- [ ] Tumor Transplantation/Induction
- [ ] Toxicity Testing (LD-50) *(Complete **Attachment G, DEATH AS AN ENDPOINT**)*
- [X] Teaching or Training Protocol *(If checked, complete Question 12a below)*

12a If this is a teaching or training protocol, please check all that apply:

- [ ] Undergraduate or graduate students
- [ ] Continuing education students (M.D.)
- [X] Only dead animals or tissues obtained through euthanasia by the PI to be used
- [ ] Demonstration (PI only performing procedures)
- [ ] Student involvement (students performing/assisting in procedures)
- [ ] Use within a Biomedical Sciences Course (ID #/Name: _____)
- [ ] Other (**Explain below**):

F. RESEARCH DESIGN:

In generating the research design, note that the reviewers are scientifically knowledgeable, however, they may not be experts in your specific field of study. Please provide a brief (one or two paragraphs) overview of the project design and how each experimental goal relates to the project design. The descriptions should provide a sequential overview of all procedures and should account for each subject (by experimental group). The overview should be followed by a chronological description of all experimental procedures related to the care and use of the animals. **The use of tables and flow charts to organize the procedures, numbers of animals, and schedules is recommended.** Do not paste in method sections from grant applications or journal articles. Do not include methods pertaining to *in vitro* work, unless it applies to the care and use of animals. For each animal or experimental group, provide information on the duration of each procedure (*i.e. fluid or tissue collections, methods, sites, volumes/weights, frequencies, etc.*) and the total time from initial contact to completion. **Although procedures involving drug manipulations and surgery are detailed in other sections of this form, their application in the research design should be stated here. Any procedures not covered in later sections of this form must be completely detailed in this section.**

By reading only this section of the *Initial Review Form*, the IACUC should be able to clearly determine each experiment being performed on each individual animal.

General overview of the project:

The overall goal of the project is to elucidate the role of estrogen in primate pregnancy on development of the fetus/placenta and impact on physiologic processes in the offspring. Over the past 30 years this laboratory using the baboon as a model for human pregnancy, has shown that estrogen is a key hormone important for placental and fetal development. Moreover, our studies have shown that critical organ systems as well as metabolic processes in the fetus appear to be programmed by estrogen, consistent with the new prevailing theory that in addition to our genetic makeup, who/what we are physiologically as adults is established *in utero* by epigenetic mechanisms (e.g. programming). Thus, interference with this intrauterine programming either by premature birth, poor fetal growth, exposure to environmental factors that interfere with and or enhance estrogen action increase the risk for development in adulthood of diseases such as hypertension and diabetes. Thus, it is critical to understand what estrogen is doing. However, in examining the role of estrogen it is important to recognize that the source and levels of this hormone change during pregnancy. During the first trimester (days 1-60 in the baboon; term = 184 days), the maternal ovary is the source of estrogen and maternal (as well as fetal) estradiol levels are typically relatively low and more like that during the follicular phase of the mother's menstrual cycle (i.e. <300 pg/ml). At the end of the first trimester, the placenta takes over and becomes the source of estradiol. As a result, the maternal (and fetal) levels of estradiol increase daily throughout the second half of gestation and by term exceed 5,000 pg/ml (fetal estrogen levels are about 20% of those in the mother). Based on our studies and proposed new experiments, too much estrogen early in gestation (e.g. as can occur in '*in vitro*" pregnancies; exposure of mother to estrogen like-molecules in the environment) or interference with the availability/action of estrogen during the second half of gestation (exposure to environmental inhibitors of the estrogen receptor; premature delivery) are equally harmful to placental/fetal development and physiologic function in adulthood.

STUDY I: ROLE OF ESTROGEN IN EARLY GESTATION

During the period of relatively low estrogen, numerous events occur that are essential for establishment of a successful pregnancy. Notably, the placenta and fetus must develop blood vessels, gain accessibility to nutrients in mother's blood and coordinate/regulate blood flow. To accomplish these things, cells in the newly developing placenta, specifically the extra villous trophoblast (EVT) migrate and attach to the uterine spiral arteries that supply mother's blood to the uterus and products of conception. These placental cells erode about 80%-90% of the smooth muscle that comprise the blood vessels. Moreover, about 50%-70% of the vessels are "invaded" by the placental cells a process termed remodeling and which renders these vessels unable to contract (e.g. when mom gets anxious/ stressed). Thus, blood just dumps into the placental space and serves as a reservoir of nutrients for the fetus. The vessels offer no resistance to flow and as a result do not significantly influence mother's blood pressure. This critical process is essentially over by the start of the second trimester. But what regulates this and why does it end at this time as there are still lots of vessels not invaded. This is a critical question because we now know that in women who develop the pregnancy complication, preeclampsia, there is "shallow placental invasion", i.e. the mother's uterine arteries are not adequately invaded by the placental cells. Preeclampsia is life threatening and often complicated by increased maternal blood pressure and reductions/complications in placental and fetal blood flow that severely restrict fetal growth. During the previous project periods, we showed that by simply injecting the baboon mother in the first trimester with estradiol and increasing estrogen levels to those normally seen at the beginning of the second trimester, we inhibited placental production of vascular endothelial growth factor (VEGF), blocked placental invasion of the uterine arteries, and disrupted placental/fetal blood flow and response of the fetal-placental vessels to the vasoactive agent serotonin. In the current project period we propose experiments to determine the mechanism by which estrogen elicits these effects and ascertain impact on placental and fetal development and whether changes in fetal blood flow persist into adulthood and thus were programmed *in utero*.

Experimental Treatment Groups:

Role of estrogen on uterine artery remodeling and placental/fetal development

Group 1: Untreated on days 25-59 (n = 8)
Group 2: Maternal estradiol daily on days 25-59 (n = 8)

Pregnant baboons will be either untreated or injected daily on days 25-59 of gestation (term = day 184) with estradiol benzoate (35 µg/kg BW/1 ml sesame oil; sc) and a maternal blood sample (2-4 ml) obtained from a peripheral saphenous vein under ketamine anesthesia (10-15 mg/kg BW). On day 60, baboons are prepared for surgery as described below and the placenta and fetus delivered and tissues isolated and stored for subsequent study of estrogen on placental angiogenesis and vascular invasion.

Groups 3-7: Untreated days 25-53; delivery of placenta and fetus on day 54

Group 3: 2 hours after acute injection of estradiol (n = 8)
Group 4: 6 hours after acute injection of estradiol (n = 8)
Group 5: 24 hours after acute injection of estradiol (n = 8)
Group 6: 48 hours after acute injection of estradiol (n = 8)
Group 7: 48 hours after acute injection of saline (n = 8)

Pregnant baboons will be untreated and a maternal blood sample (2-4 ml) obtained from a peripheral saphenous vein under ketamine anesthesia (10-15 mg/kg BW) on days 25-53 of gestation. Placentas and fetal tissues will be obtained by cesarean section on day 54 of gestation 2h (Group 3), 6h (Group 4), 24h (Group 5), or 48h (group 6) after an iv bolus injection of 1 µg/kg BW 17β-estradiol in 1.0 ml saline: 5% ethanol and a sc injection of estradiol benzoate (25 µg/kg BW in 0.2 ml sesame oil) or vehicle alone (Group 7). Animals in Group 6 will also be administered a sc injection of estradiol benzoate (25 µg/kg BW in 0.2 ml sesame oil) at 24 hours. Baboons are prepared for and surgery completed as outlined in detail below and the placenta and fetus delivered and tissues isolated and stored for subsequent study of estrogen on placental angiogenesis and vascular invasion. These acute studies will permit us to ascertain estrogen mechanisms and whether estrogen is acting directly or indirectly.

Estrogen suppression of uterine artery remodeling in early pregnancy: impact on cardiovascular dynamics at term and in the offspring

Group 8: Untreated on days 25-59; studies on day 160-170 (n = 8)
Group 9: Maternal estradiol on days 25-59; studies on day 160-170 (n = 8)

Pregnant baboons will be either untreated or injected daily on days 25-59 of gestation with estradiol benzoate (35 µg/kg BW /1 ml sesame oil; sc) and a maternal blood sample (2-4 ml) obtained from a peripheral saphenous vein under ketamine anesthesia (10-15 mg/kg BW). Maternal peripheral saphenous blood samples (2-4 ml) will then be obtained at 5-day intervals and animals not treated further. On day 160-170, uteroplacental blood flow dynamics during infusion of saline or serotonin at 4 µg/kg BW and then 8 µg/kg BW will be determined by color Doppler ultrasonography and the placenta and fetus then delivered by cesarean section as outlined below and tissues isolated for subsequent study of estrogen on placental and fetal development.

Group 10: Untreated days 25-59; deliver spontaneously; study offspring (n = 8)
Group 11: Maternal estradiol on days 25-59; deliver spontaneously; study offspring (n = 8)

Pregnant baboons will be either untreated or injected daily on days 25-59 of gestation with estradiol benzoate (35 µg/kg BW /1 ml sesame oil; sc) and a maternal blood sample (2-4 ml) obtained from a peripheral saphenous vein under ketamine anesthesia (10-15 mg/kg BW). Baboons will be left untreated throughout the remainder of pregnancy and allowed to spontaneously deliver neonates at term. These studies will allow us to ascertain whether vascular changes induced *in utero* persist in adulthood and thus were programmed *in utero*.

Briefly, babies are examined daily by Dr. Pepe's staff and weaned from their mothers at 8-12 months of age. At 2-4 week intervals beginning at approximately 6 months of neonatal age, mothers are sedated with ketamine and all neonates removed, sedated with ketamine, weighed, a gross physical examination performed by PI staff and blood samples (1-3 ml) obtained for determination of blood chemistries (e.g. Na, K, glucose) and hormone profiles (cortisol, androgens, estradiol; insulin, prolactin, growth hormone, ACTH).

Starting at 8 months of age, we propose to measure cardiovascular dynamics including blood pressure, heart rate and brachial artery diameter and flow before and after occlusion (of the brachial artery) by ultrasonography/Doppler as well as microvascular flow and density using contrast enhanced ultrasonography during infusion of saline and then infusion of serotonin (or phenylephrine or nitroprusside or acetylcholine). At least one month (30-45 days) later these studies are repeated but the animal infused nitroprusside (or phenylephrine or serotonin or acetylcholine). At least one month (30-45 days) later the analyses are repeated but phenylephrine (or nitroprusside or serotonin) infused such that each animal receives sequentially all 4 agents but in a randomized manner. In addition, prior to infusion of the second agent we propose to collect a biopsy of skeletal muscle for biochemical analyses. The entire protocol is then repeated during developmental age period 18-24 months and then again at developmental age 36-48 months of age. The description of the protocol outlined below includes a section on infusion of vasoactive agents e.g. dosages etc, a description of the cardiovascular function tests to be performed by ultrasound including the analysis of microvascular flow by contrast enhanced ultrasound, and a description of the skeletal muscle biopsy.

Total number of pregnancies for Study I: N = 88 (8/group x 11 groups)

STUDY II: ROLE OF INCREASING LEVELS OF ESTROGEN DURING THE SECOND HALF OF GESTATION

During the second half of pregnancy in humans and baboons, there is a tremendous increase in estrogen production by the placenta accompanied by significant growth, differentiation and maturation of the fetus and the placenta. Our laboratories have shown that this increase in estradiol is essential for structural and functional maturation of the placenta (e.g. microvilli; enzymes controlling cortisol metabolism) and organ systems in the fetus including the fetal ovary and adrenal gland. Moreover, estrogen also appears to program tissues/organ systems of the fetus that impact insulin sensitivity as well as reproductive function. Interest in estradiol in pregnancy is heightened as mentioned above by studies showing that factors which interfere with the availability or action of estradiol increase risk for development of disease including diabetes in adulthood. Our overall goal is to ascertain the mechanisms by which estrogen regulates development of the primate fetal adrenal gland, development of insulin sensitivity and the formation of the pool of ovarian follicles comprised of healthy oocytes essential for reproductive function in adulthood. To test our hypotheses, we treat pregnant baboons with an aromatase inhibitor (letrozole) without/with estrogen to reduce/restore estrogen production during the second half of gestation and remove fetal tissues, e.g. ovaries and adrenal glands as well as skeletal muscle, fat and the pancreas to study expression of proteins that are essential for insulin action. *In vitro* studies are also performed to determine the mechanism of estrogen action including incubation of fetal adrenal cells with ACTH in presence/absence of estradiol or the estradiol receptor agonists and/or inhibitors of downstream signaling molecules. We also examine maternal glucose homeostasis (e.g. glucose-tolerance tests; fasting insulin) and other hormones/factors (e.g. androgens, cortisol, cytokines) to confirm that alterations in the fetal adrenal, ovary or fetal tissue glucose tolerance/insulin sensitivity are not the result of modification of maternal mechanisms. Finally, neonates born to mothers treated *in utero* with nothing, aromatase inhibitor ± estrogen are raised to adulthood (puberty at 40-48 months of age) and adrenal and ovarian function as well as glucose tolerance/insulin action and microvascular blood flow by Doppler contrast enhanced ultrasound studied prior to and after onset of puberty to determine impact of estrogen programming of fetal adrenal, metabolic systems and ovarian development on adult reproductive, metabolic and adrenocortical function.

Experimental Treatment Groups:

Estrogen regulation of placental development, fetal adrenal maturation and ovarian follicular formation and programming of fetal tissue sensitivity to insulin

Group 1: Untreated days 80-100 (n = 16; 8 male ♂/8 female ♀ fetuses)

Group 2: Untreated days 80 to 160-170 (n = 16; 8 ♂/8 ♀ fetuses)

Group 3: Treated with letrozole; days 100 to 160-170 (n = 16; 8 ♂/8 ♀ fetuses)

Group 4: Treated with letrozole + estradiol; days 100 to 160-170 (n=16; 8 ♂/8 ♀ fetuses)

Briefly, female baboons within our primate colony are mated with male baboons of proven fertility, pregnancy confirmed by ultrasound and fetal sex determined by chromosomal analysis of fetal cells isolated from amniotic fluid obtained at day 80 of gestation (term = day 184). Baboons are then allocated to one of the four treatment groups and thus are either untreated or treated daily on days 100 to 160-170 of gestation with letrozole (115 µg/kg BW/day/1.0 ml sesame oil; sc) or letrozole plus estradiol benzoate (both 115 µg/kg BW) administered sc to the mother. Maternal saphenous blood samples (2-4 ml) are obtained from ketamine-sedated baboons every 2-4 days beginning on day 80 and from a maternal uterine vein (5 ml) and umbilical vein (5 ml) and artery (4-8 ml) at the time of cesarean section on days 100 or 160-170 for RIA analysis of serum levels of steroid (e.g. estradiol) and protein (e.g. ACTH) hormones. In addition, two maternal iv glucose tolerance tests will be performed during the study period, one just before (between days 80 and 120 of gestation) and the second late in gestation (days 150-160) and thus after onset of/during treatment with nothing or letrozole ± estradiol. These studies are essential to establish potential effects of treatment on maternal physiology that could impact interpretation of findings of the role of estrogen on the fetus. Animals are then delivered by cesarean section, fetuses euthanized and placental and fetal tissues (e.g. ovary/testes; adrenals; liver, lung, heart, kidney; brain, pituitary, skeletal muscle; adipose; pancreas) frozen or formalin-fixed.

Estrogen programming of the baboon fetal adrenal, ovary and skeletal muscle *in utero* determines adrenocortical, reproductive and metabolic function and fertility in adulthood

Group 5: Untreated days 100-175; study offspring (n = 16; 8♂/8♀)

Group 6: Letrozole on days 100-175; study offspring (n = 16; 8♂/8♀)

Group 7: Letrozole plus estradiol; days 100-175; study offspring (n = 16; 8♂/8♀)

Briefly, after determination of fetal sex by chromosomal analysis of fetal cells isolated from amniotic fluid obtained at day 80 of gestation, baboons are allocated to one of the three treatment groups and thus are either untreated or treated daily on days 100 to 175 of gestation with letrozole (115 µg/kg BW/day/1.0 ml sesame oil; sc) or letrozole plus estradiol benzoate (both 115 µg/kg BW) administered sc to the mother. Maternal saphenous blood samples (2-4 ml) are obtained from ketamine-sedated baboons every 2-4 days beginning on day 80 and in addition, two maternal iv glucose tolerance tests performed during the study period, one just before/during (i.e. between days 80 and 120 of gestation) and the second late in gestation (days 150-160) and thus after onset of treatment with nothing or letrozole ± estradiol. Neonates are delivered spontaneously or by cesarean section at term (day 184) and reared to adulthood. We have considerable experience hand rearing baboon neonates and found that offspring either nursed/raised by their mothers or fed/raised by staff exhibit very similar behavior, rates of postnatal growth/body weight and basal

levels of glucose, insulin and serum chemistries.

Babies are examined daily by Dr. Pepe's staff and weaned from their mothers at 8-12 months of age. At 2-4 week intervals beginning at approximately 6 months of neonatal age, mothers are sedated with ketamine and all neonates removed, sedated with ketamine, weighed, a gross physical examination performed by PI staff and blood samples (3 ml) obtained for determination of blood chemistries (e.g. Na, K, glucose) and hormone profiles (cortisol, androgens, estradiol; insulin, prolactin, growth hormone, ACTH). The following studies will be performed prior to and after onset of puberty: [1] Intravenous glucose tolerance testing; [2] brachial arterial blood flow by Doppler ultrasound and microvascular flow/density by contrast enhanced ultrasound during infusion of physiologic saline (control), serotonin, sodium nitroprusside, acetylcholine or phenylephrine. [3] determination of onset of puberty defined as onset of regular menstrual bleeding; [4] adequacy of ovarian function and thus analysis of estradiol and progesterone serum levels during the menstrual cycle; briefly, animals are sedated with ketamine and blood samples (3 ml; total blood sample volume <10% total blood volume/not to exceed 10 ml/kg/month) obtained at 1-2 day intervals during two subsequent menstrual cycles (cycle one animal studied; cycle two animal not studied; cycle three animal studied) and ovarian follicle size and uterine endometrial growth determined by non-invasive ultrasound; a total of 8 ultrasounds are performed e.g. before, at and after ovulation; [5] Baboons, now adults are then examined for fertility, i.e. ability to achieve (mated with males of proven fertility) and maintain pregnancy and deliver a healthy neonate. [6] animals are hemi-ovariectomized (as outlined below) to assess ovarian follicle reserve/biochemistry/oocyte health; [7] *in vivo* studies to examine the pituitary-adrenal axis i.e., serum cortisol and androgen levels following acute infusion of ACTH (or saline vehicle) or ACTH (or saline vehicle) 48h following treatment with betamethasone to suppress the pituitary-adrenal axis; [8] *in vivo* analysis of brainstem auditory evoked response (a non-invasive procedure to examine hearing); [9] intravenous glucose tolerance tests and analysis of brachial flow by Doppler analysis and microvascular flow by contrast enhanced ultrasound during infusion of saline (control), serotonin, sodium nitroprusside, acetylcholine, or phenylephrine; these studies are a continuation of those performed in the prepubertal and early postpubertal period, i.e. into adulthood to test progression of physiological function.

Total number of pregnancies for Study II: N = 112 (16/group x 7 groups)

Overall total number of pregnancies: N = 200 (88 for Study I and 112 for Study II)

This research program continues to function as a collaborative effort with colleagues at the University of Maryland as has occurred over the past 30 years. Thus, approximately 50% of the studies/animal treatments will be performed at the University of Maryland and 50% at EVMS and tissue samples shipped between Institutions. Therefore, ***a total of 100 pregnancies are required at EVMS. As treatments are associated with a 15% incidence of spontaneous abortion, a total of 115 pregnancies (or 23 pregnancies/year over a 5-year period) are required to complete the objectives outlined.*** Based on our experience and multiple use of baboons (control, estrogen suppression, estrogen treatment etc), a population of 28 adult female and 5 adult male baboons (proven breeders) is required to meet the objectives of this study. Because multiple surgeries are limited, we also have determined that we need to purchase at least 3 and up to 5 adult female baboons yearly to "turn over" the colony.

HUSBANDRY:

Juvenile and adult female baboons are housed in USDA regulated cages. Socialization and behavior is monitored by PI staff and CompMed jointly. When possible, female baboons are socialized and pair housed with compatible females. Some pairs are fully open allowing free interaction. In some cases as a result of aggressive behavior causing injury or other negative physical conditions, two females are 'partial paired' meaning they do not have continues free interaction but are restricted allowing tactile contact and socialization on a limited level. There are some animals which cannot successfully be paired to any level. All animals are housed in rooms with multiple other animals allowing for vocal and visual stimuli. Cycling adult female baboons are paired with male baboons for breeding purposes 5 days prior to ovulation as determined by perineal turgescence or sex skin swelling. Successful breeding resulting in pregnancy is confirmed by ultrasound on day 25 (day 0 = day of ovulation) and/or lack of menstrual cycle and halted sexual skin swelling.

SURGERIES:

Cesarean section: On varying days of gestation based on the study group, baboons are briefly restrained in home cage via squeeze mechanism, injected with ketamine (10-15 mg/kg), intubated, anesthetized with isoflurane/ oxygen and vitals (e.g. heart rate, blood pressure) monitored by CompMed staff. A 22g catheter is placed in the brachial vein and IV fluids administered (Lactated Ringers Solution or 0.9% saline). A second catheter is placed in the saphenous vein using a 19g catheter 24inches in length and IV fluids administered (1.6ml/min/90min). The animal's abdomen/ surgical site is shaved and scrubbed with alcohol and Betadine solution. The animal is draped using sterile technique. Blood samples (3ml) are obtained from the mother at '0' time, mid procedure and post placental delivery via saphenous catheter. Prior to surgery, a proper plane of anesthesia will be assured by lack of response to a noxious stimulus such as toe pinch. A vertical mid-line incision is made using a 10blade. The incision is 4-12cm in length depending on gestational age. Once the abdomen is open, the uterus is externalized and examined for placenta location. Mother's ovaries are examined for evidence of ovulation/corpus luteum. Two to four uterine vein blood samples are taken (5 ml) using a 23g needle. Fetal crown is localized and a horizontal incision is made in the uterus to remove the fetus. Amniotic fluid is recovered using a syringe. Once the fetus is carefully exteriorized, umbilical vein

and artery samples are taken using a 23g butterfly set (4-8ml) for hormone, steroid and blood gas analysis. The umbilical cord is double clamped to ensure the safety of the mother; the fetus is then euthanized by injecting via the umbilical artery Fatal Plus euthanasia solution. In the event the umbilical artery is not an adequate route for euthanasia, a cardiac stick will be used only when necessary. After the fetus expires (no heart beat), the cord is cut, placenta is delivered. Segments of the placenta and the fetal adrenal, hypothalamus, pituitary gland, lungs, kidneys, liver, skeletal muscle, pancreas, heart and regions of the brain subcutaneous and visceral fat, and gonads are collected, portions fixed in formalin or snap frozen for subsequent immunocytochemical-biochemical/ mRNA determinations. The uterus is cleaned and closed using absorbable suture. The uterus is manually messaged to stimulate contractions and shut down bleeding. Once closed, the uterus is rinsed with sterile saline and placed back in the abdomen. The abdomen is then rinsed with sterile saline to remove any blood clots. Prior to closing the abdomen, a small 10-15gram sample of visceral/ abdominal fat is ligated and removed for RNA analysis. The abdomen is closed by three layers; the first layer (peritoneum) is closed using simple interrupted stitch. If present, a second layer (fascia) is closed using absorbable suture. If a clear fascia layer is not present then a SQ layer is closed using a continuous stitch. The skin is then closed with absorbable suture. Vet-bond adhesive glue is applied to the incision line once skin is closed. The mother is injected with Iron Dextran IM (10 mg/kg BW) for iron supplementation. Isoflurane is shut off and animal is monitored until swallowing is present. Once able, the animal is extubated and continues to be monitored in home cage until fully upright and responsive to stimuli. Immediate post-op monitoring is done by Com Med staff using procedures outlined in the EVMS Post-Operative Care Guidelines Manual and in Attachment E below. PI staff is also present during immediate recovery. In selected experiments, the fetus is delivered live either spontaneously (on day 184) or by cesarean section on day 170 and reared to adulthood (also please see experimental treatment group #12; page 15).

Terminal cesarean section: Surgery is followed as outlined above. Once the fetus is euthanized and cord is cut, the mother is given an IV injection Fatal Plus (1ml/4.5 kg). Once HR and respiration has ceased, the abdomen I opened to harvest tissues (liver, lung, adrenal, ovary, pancreas, muscle and fat).

Hemi-ovariectomy: After completion of puberty and fertility studies, adolescent baboons from experimental group #8 (approximately 6-10 years of age) are briefly restrained in home cage via squeeze mechanism, sedated with ketamine (10-15mg/kg), intubated, anesthetized with isoflurane/oxygen and vitals (e.g. blood pressure) monitored by CompMed staff. A 22g catheter is placed in the brachial vein and IV fluids administered (Lactated Ringers Solution or 0.9% saline). A baseline blood sample (5-8ml) is taken to check blood gas levels and run hormone analysis. The animals' abdomen/surgical site is shaved and scrubbed with alcohol and Betadine solution. The animal is draped using sterile technique. Prior to surgery, a proper plane of anesthesia will be assured by lack of response to a noxious stimulus such as toe pinch. After making a small abdominal incision (approximately 6-10cm) a retractor is put in place, lap sponge can be used if needed for a clearer view, ovaries are located. Once the ovulation site is located, the ovarian ligament and vasculature is identified, clamped and cauterized and the ovary removed. Once removed, clamps are carefully removed and ligament/cautery site observed for bleeding. Abdomen is rinsed with sterile saline. Abdominal layers are closed (peritoneum, fascia/SQ and skin) using 2-0 PDS and 2-0 Dexon. Vet-bond skin adhesive applied to the incision site once closed. Isoflurane is shut off and animal is monitored until swallowing is present. Once able, the animal is extubated and continues to be monitored in home cage until fully upright and responsive to stimuli. Immediate post-op monitoring is done by Com Med staff using procedures outlined in the EVMS Post-Operative Care Guidelines Manual and in Attachment E below. PI staff is also present during immediate recovery. Animals will be treated postoperatively with analgesics and recovery monitored as outlined in the Attachment E below.

Terminal biopsy: As a terminal procedure for those adult EVMS reared baboons which have completed all survival studies as outlined, a terminal biopsy procedure to collect skeletal muscle, adipose tissue and pancreatic samples will be performed as follows: Baboons are sedated with ketamine (10-15mg/kg) and prepped / monitored for the procedure under CompMed SOP. A baseline blood sample (5ml) is obtained from the saphenous vein using a 21g needle to determine blood chemistry, gases and acid/base status and subsequent analysis of estradiol. Progesterone and androgens. HR, BP, respiratory rate, O_2 saturation and body temp are monitored by Comp Med; a surgical plane of anesthesia is maintained. A 2-3 cm incision is made in the bicep to expose the muscle. A section is removed using a 3-6mm punch biopsy. The incision is secured using a clamp. A second incision is will be made from the xiphoid process to the pubis. A section of abdominal and subcutaneous fat will be excised. Finally, the pancreas is isolated using blunt dissection. One biopsy is taken from the tail region. The pancreas is then completely removed for further sampling. The surrounding vessels are clamped immediately to control bleeding. As soon as the pancreas is free, Fatal Plus (Pentobarbital Solution) euthanasia solution is administered (1ml/4.5kg) IV followed by a saline flush. Euthanasia is confirmed by the absence of HB and flat ECG. Subsequent FP will follow PRN.

Amniocentesis for sex determination: Baboons with fetal gestation approximately 80-95days are sedated with ketamine and placed on Isoflurane/O2 via cone mask. Fetal/placental position is determined using GE Logic+ ultrasound. Using a 18g x 2in needle, 10ml of amniotic fluid is removed from the uterus. The fetal HR is rechecked and 3ml of blood taken from maternal saphenous vein using a 21g needle. The animal is turned off anesthesia and returned to home cage to be monitored until sitting upright. The entire procedure takes less than 10 minutes.

Intravenous glucose tolerance test (IVGTT): An IVGTT will be performed in adolescent baboons at from 12 to 72 months of age (i.e. before, during and after onset of puberty which occurs at approximately age 40 ± 1 month) once

every 6 months and pregnant untreated and treated animals on days 80-120 and 155-165 days gestation (minimum 4 weeks between the two test) Briefly, baboons (4-20 kg body weight) are fasted overnight, sedated with ketamine (10 mg/kg), a catheter inserted into a peripheral saphenous and an antecubital vein and a 250ml 0.9% sterile saline drip with 500mg ketamine is initiated. Two initial base line blood samples are taken and blood glucose and blood gas levels determined. At experimental time zero, a bolus of glucose (0.25 grams/kg BW; 0.05 ml 50% dextrose/kg BW; Abbott Laboratories) is injected into the antecubital vein. Blood samples (total blood sample volume < 10% total blood volume/not to exceed 10ml/kg/month) are collected into from the saphenous catheter at '0', 1, 3, 5, 10, 20, 40 and 60 mins. During the experiment, the animal is monitored for BP, HR, respiration, and warmed via Warming blanket. At completion of the experiment, animal is given 1cc (10mg/kg) iron dextran IM. Animals are returned to their cages and monitored to recovery to stage 0.

Brainstem auditory evoked response in control and Letrozole/Letrozole + estradiol-treated animals: Baboons pre and post pubertal will be sedated with ketamine, a catheter placed into a antecubital vein and NaCl drip (approximately 10ml/kg/hr) initiated and placed on Isoflurane/O2 via a cone mask. HR and respiratory rate will be monitored. A small pathc of hair is shaved above the brow, at the vertex and mastoids then small (~22mm) electrodes placed subcutaneously. BAER will be evoked and recorded using a Biologic Navigator Pro system by monaural stimulation of the ipsilateral ear at 80 dB hearing level. Alternating clicks (n= 2000) of 0.15 ms duration will be presented at a rate of 27.7/sec. Band pass filtering will be set at 100-3000Hz. In addition we will test low frequency hearing by presenting tone bursts of 80dB intensity at 500 and 1000Hz. The protocol for the tone bursts will have 1500 sweeps, a rate of 39.1/sec, a duration of 8ms, and a band pass filtering of 30-1500Hz. Once initiated, these recordings are expected to be completed over a period of 25 minutes. During the procedure, animals are kept warm (on a heat controlled BEAR hugger and heated table) and blood pressure, heart rate, respiration, oxygen/ saturation monitored. At completion of each experiment, baboons are returned to their cages and monitored to recovery.

Pituitary/adrenal axis in 7+year old EVMS born baboons: Post pubertal baboons from study group #8 are studied in two groups.

Study 1: Response to ACTH: Baboons (weighing 11-18 kg) are fasted overnight and sedated with Ketamine, HCl (10-15mg/kg), and a catheter inserted into a peripheral saphenous vein for blood sampling and infusion of NaCl (250 ml) containing 500 mg ketamine and a second catheter (AngioCath; 23 gauge needle) inserted into the antecubital vein for drug administration. Following a 10 min stabilization period, blood samples (3.0 ml) are obtained from the saphenous vein at -10 and 0 min. A time '0' acute bolus of saline (0.2 ml) alone or saline and ACTH (Cortrosyn; 1.0 µg/0.2 ml saline) is injected via the antecubital vein catheter. Saphenous blood samples are taken at +15, +30, +45 and +60 min. At +60 minutes a second bolus of ACTH (Cortrosyn, 10.0 µg/0.2 ml) is injected and saphenous blood samples obtained at +75 +90 and +120 min (total blood sample volume < 10% total blood volume/not to exceed 10ml/kg/month). Each animal will be studied twice (saline alone or ACTH bolus) during a 2 month period.

Study 2: Response to Betamethasone/ACTH: Approximately 2-3 months after completion of Study 1, the ACTH protocol will be performed in baboons treated. Briefly, at -24 and -3h prior to ACTH response, animals are sedated with ketamine and blood samples (3 ml) obtained at the time of im injection of betamethasone (0.3 mg/kg BW; Celestone Soluspan). Animal is sedated 3hours later and ACTH response study initiated as in Sturdy 1. See chart below. For the conduct of both Studies 1 and 2, animals are monitored for HR, BP, spO_2, blood gas levels and warmed via bear hugger. At completion of each experiment, baboons are injected with 1ml iron dextran, returned to their cages and monitored until upright.

Time	Betamethasone	Blood sample	Blood gas	ACTH/Saline	Iron Dextran
Study 1 – Response to ACTH					
Time '0'		Yes	Yes	Yes	
Time 15min		Yes			
Time 30min		Yes			
Time 45min		Yes			
Time 60min		Yes		Yes	
Time 75min		Yes			
Time 90min		Yes			
Time 120min		Yes	Yes		Yes
2-3 months later					
Study 2 – Response to Betamethasone / ACTH					
Time -24hrs	Yes	Yes			
Time -3hrs	Yes	Yes			
Time -10min		Yes			
Time '0'		Yes	Yes	Yes	
Time 15min		Yes			
Time 30min		Yes			
Time 45min		Yes			
Time 60min		Yes		Yes	
Time 75min		Yes			
Time 90min		Yes			
Time 120min		Yes	Yes		Yes

Effects of pharmacological agents on cardiovascular function and vessel response in pregnant baboon: [Study group 3 only] pregnant baboons are treated with nothing or estradiol (350 µg/kg maternal body wt) daily on days 25-59 of gestation, animals not further treated and then delivered at day 165-170 of gestation (term = day 184) or allowed to spontaneously deliver at term and raise the neonate. Using this treatment paradigm we have shown that a premature elevation in estradiol early in pregnancy regulates uterine spiral artery invasion and have proposed that the latter impacts utero-placental blood flow dynamics and blood flow/cardiovascular function in the neonate/adolescent. To test this hypothesis, we propose to determine basal (resting) and serotonin-induced uterine arterial and umbilical (fetal) arterial and fetal middle cerebral arterial blood flow dynamics as well as fetal heart rate using Doppler analysis at days 60, 100 and 165 of gestation and basal and serotonin-induced cardiovascular function in the neonate at 3, 6, 12, 24, 36 and 48 months of age. For intrauterine studies, baboons are sedated with ketamine (10-15mg/kg im) and prepped/monitored for the test under Comp Med SOP. An intracatheter is placed in the maternal saphenous vein with 0.9% NaCl drip at 1ml/min; a baseline blood sample (3 ml) obtained to determine blood chemistries (Na, K, etc), gases (pCO_2, pO_2) and acid/base status and subsequent analysis of estradiol, progesterone and androgens. Maternal heart rate, blood pressure and body temp are monitored by Comp Med throughout the procedure. Briefly, animals are infused with saline (0.5 ml/min) for 20 mins and fetal heart rate measured/monitored continuously and uterine, umbilical, and fetal middle cerebral arterial blood flow dynamics determined during the final 5 mins of infusion using Ultrasound/Doppler procedures. After collection of basal data, a maternal infusion of serotonin (4 µg/kg/min) is initiated and fetal heart continuously measured and blood flow/chemistry studies performed during the final 5 mins of this 20 min infusion. Upon completion, the dose of serotonin is increased to 8 µg/kg BW/min and blood flow/chemistry analyses determined as described. Infusion of serotonin will be stopped immediately should fetal heart rate decrease to 80 bpm. If fetal HR drops below 80 bpm for more than 3 minutes, terbutaline will be administered IV/SQ to the mother under direction of the attending veterinarian to alleviate the fetal bradycardia. If fetal HR does not return to normal and continues to drop or fetal demise occurs, a cesarean section will be performed as outlined. Once the final dose of 5-HT is infused, the animal is monitored until recovered under Comp Med post op monitoring SOP and returned to home cage. The mother is being monitored for HR, BP and body temp throughout the procedure.

Effects of pharmacological agents on cardiovascular function and vessel response in the neonate: Briefly, starting at 8 months of age, we propose to measure cardiovascular dynamics (blood pressure, heart rate and brachial artery diameter and flow before and after occlusion and microvascular flow using contrast enhanced ultrasonography during infusion of saline and then infusion of serotonin (or phenylephrine or acetylcholine or nitroprusside). At least one month (30-45 days) later these studies are repeated but the animal infused nitroprusside (or phenylephrine or serotonin or acetylcholine). At least one month (30-45 days) later the analyses are repeated but phenylephrine (or nitroprusside or serotonin or acetylcholine) infused. The study is repeated again one month (30-45 days) later but the animal infused acetylcholine (or nitroprusside, serotonin or phenylephrine) such that each animal receives sequentially all 4 agents but in a randomized manner. In addition, prior to infusion of the second agent we propose to collect a biopsy of skeletal muscle for biochemical analyses. The skeletal muscle biopsy is collected twice during the entire period of neonatal development at 8-14 months of age and then again at 18-24 months.

The entire protocol is then repeated during developmental age period 18-24 months and then again at developmental age 36-48 months of age. The description of the protocol outlined below includes a section on infusion of vasoactive agents e.g. dosages etc, a description of the cardiovascular function tests to be performed by ultrasound including the analysis of microvascular flow by contrast enhanced ultrasound, and a description of the skeletal muscle biopsy.

Infusion of Vasoactive Agents: During the infusion, baboons will be placed in a supine position upon a Bear Hugger to maintain body temperature. Baboons will be sedated with ketamine (10-15 mg/kg bw, IM, 23 gauge needle) and anesthetized with isoflurane and receive an infusion of 0.3 ml saline/min via a sterile 21-gauge catheter inserted into an antecubital or saphenous vein and then an infusion of serotonin at 4 and 8 µg/kg/min/0.3 ml saline or phenylephrine at 1 and 5 µg/kg bw/min/0.3 ml saline or of nitroprusside at 1 and 3 µg/kg bw/min/0.3 ml saline, or of acetylcholine (2 and 4 µg/kg bw/min/0.3 ml saline) for 20-30 min per dose to permit the flow measurements described below. Each agent (pharmaceutical grade except for serotonin and acetylcholine) will be dissolved in 0.9% sterile saline. The maximum dose of phenylephrine (5 µg/kg bw/min) proposed in the baboon is well within the range (10 µg/kg bw/min) that is administered to humans and which does not elicit excessive hypertension or tachycardia. The maximum doses of nitroprusside (3 µg/kg bw/min) and acetylcholine (4 µg/kg bw/min) proposed in the baboon, are the average dose infused to humans and which do not cause excessive hypotension, bradycardia or cyanide toxicity (NIH Daily Med Search; Medicine Online; Reed et al., Am J Physiol E472, 2004). Another 21-gauge catheter will be inserted into the other antecubital or saphenous vein, or femoral vein and blood samples (3 ml) obtained prior to and at the end of infusion of the final dose of vasoactive agent to assess $P0_2$ and $PC0_2$ levels (via I-STAT analytical cartridge System, Abbott Labs) and markers of endothelial function (e.g. nitric oxide). Arterial blood pressure and heart rate will be continuously monitored by comp med and recorded in the health record. *Once a steady state HR, BP is determined, if a continual plane of elevation or depression is seen in response to the infusion, the infusion will be stopped and if needed, corrective action taken under the direction of the veterinarian.*

Cardiovascular Function by Ultrasonography: Noninvasive assessment of cardiac function via echocardiography (i.e. ejection fraction, stroke volume, etc.) and renal and carotid artery flow will be performed using a GE LOGIQ eVet

ultrasound system and transducer during the infusion of saline and at each concentration of vasoactive agent. Noninvasive brachial artery flow-mediated dilation and volumetric flow will also be assessed by Doppler ultrasonography as originally approved at each concentration of vasoactive agent to evaluate indirectly systemic vascular endothelial function. Briefly, maximum diameter and volume flow of the brachial artery will be quantified at systole and diastole before and immediately after occlusion of brachial artery flow by placing a pediatric cuff around the left or right arm approximately 1-2 inches below the antecubital fossa and inflating to 50 mm Hg above average systolic blood pressure for 5 min to induce hyperemia. Immediately after assessing brachial artery flow, noninvasive quantification of microvessel flow within skeletal muscle will also be performed by microbubble contrast enhanced ultrasonography (CEU). To conduct CEU, a sterile octafluoropropane gas-filled albumin microbubble suspension (pharmaceutical grade, Definity, St. Louis, MO) in sterile 0.9% saline will be infused at 0.1 ml/min via a syringe pump via the opposing antecubital or saphenous vein catheter (from which blood is obtained) for the duration of infusion of the 0 µg and maximum doses only of phenylephrine, serotonin or nitroprusside. The linear array transducer is placed on the skin surface overlapping either the vastus lateralis muscle or lower forearm (preliminary analyses will establish which site is optimal, not same area where biopsy obtained-see below) and used to assess microvessel flow. Infusion of octafluoropropane gas-filled albumin microbubbles and CEU are routinely performed to assess microvessel flow in humans with no adverse effects (Timmerman et al., JCEM 195:3848, 2010; Lindner et al., J Am College Cardiac Imaging 1:343, 2008).

Skeletal Muscle Biopsy: Biopsy of vastus lateralis skeletal muscle will be obtained to quantify α1 adrenergic receptors and markers of endothelial cell function (e.g. endothelin-1, nitric oxide synthase) as indices of microvascular reactivity. The area is shaved and prepped using sterile technique. The animal is then covered with a sterile disposable laparotomy sheet for purposes of maintaining an aseptic field during biopsy. A small incision (≤ 0.5cm) is made through the skin with a #10 surgical blade to access the underlying skeletal muscle for biopsy. Using a 4-6mm disposable punch biopsy, a section of the muscle will be excised. Following the biopsy, hemostasis will be attained by direct pressure applied to the incision site and the incisions closed using absorbable suture. Once the final dose is infused, the animal is monitored until recovered under Comp Med post op monitoring SOP and returned to home cage. The mother is being monitored for HR, BP and body temp throughout the procedure.

14. Adverse Effects: Monitoring and Management

14a. In detail describe the potential adverse effects of each experimental procedure or agent administered to animals. For each item, include a statement detailing how the adverse effects would be clinically managed, should they occur.

Ketamine: IM injection for chemical restraint prior to all procedures including blood sampling. Ketamine is a dissociative anesthetic. Animals can develop tolerance and require increasing doses for effective sedation. Adverse effects can include nerve damage (if injection is improperly placed) and decreased appetite. Also, Ketamine can have a long term effect on kidney function. When possible, the lowest dose is used and each animal is evaluated on its responsive behavior to the drug. Also, blood gas analysis is done bi-weekly to monitor BUN levels. All changes in weight, appetite or blood chemistry are reported to veterinary staff (veterinary technicians and/or veterinarian).

Flunixin meglumine (Banamine): Banamine will be injected IM for pain management. This may cause GI upset if given for too long or overdose. If GI upset is observed, an alternate medication will be given.

Isoflurane: inhaled to maintain proper plane of anesthesia during surgical procedures, amniocentesis, BEAR and Doppler. Adverse effects: none anticipated. Animals are closely monitored during procedures. If the animal moves, shows eye movement, has increased jaw tone, or shows a rapid increase in heart rate or blood pressure, then isoflurane administration will be increased. Possible side effects can be hypotension, dose-dependent respiratory suppression, cardio depression and GI effects (nausea, vomiting, ileus).If animal shows a decreased heart rate, decreased blood pressure, or pale gum color with reduced capillary refill time (CRT), then isoflurane administration will be decreased, along with decreased intravenous fluid flow rate.

Ketoprofen: IM administration for pain relief. Long term administration can cause ulceration of the GI tract and GI bleeding; more rarely kidney damage and other bleeding disorders can occur. Adverse effects are not anticipated with the short-term administration described in this application.

Abdominal surgery: general risks associated with abdominal surgery include blood loss, infection, and adhesions. Undetected blood loss will be prevented by ensuring hemostasis before closing surgical incisions. All animals are monitored during the post-operative period (as defined by IACUC policy) for signs of internal bleeding (vaso-constriction and resulting loss of color of digits/extremities, lethargy, dehydration). Infection will be minimized by use of sterile equipment and supplies, disinfection of the incision site, performance of surgery in a dedicated surgical suite, and use of aseptic technique during the procedure. Infection rate is minimal to none in 300+ survival surgical procedures performed by the PI/PI staff at EVMS. The Veterinarian will be consulted if unusual redness, swelling, or discharge is noted at the incision site. Adhesions will be minimized by gentle manipulation of internal organs and lavage of the abdominal cavity with warm saline to remove clotted blood before closing surgical incisions.

Ovariectomy: unilateral ovariectomy results in an animal which maintains normal menstrual cyclicity; bilateral

ovariectomy will yield an infertile animal. While generally healthy, ovariectomized animals may experience physical changes associated with hormone withdrawal at menopause, including decreased bone density, weight gain, and urinary incontinence.

Amniocentesis: general side effects are stress to the fetus and possible abortion. A clear area free of fetus and placenta is localized and a sample (10ml) of amniotic fluid removed. Fetal HR is checked. Any distress noted will be discussed with the Veterinarian.

IVGTT: side effects are minimal. Possible short term anemia and depreciated appetite from ketamine. Animals are given Iron Dextran injection at completion of experiment and supplemented with children's vitamin containing iron. Also, food intake following experiment is monitored.

Dextrose (50%): local pain and vein irritation may occur. Diabetic coma, delirium tremors and congested states or pulmonary edema are unlikely but potential consequence. HR and BP are monitored before and after injection. Fetal HR is checked in the case of pregnant baboons.

Sodium Nitroprusside: relaxation of vascular smooth muscle and consequent dilation of peripheral arteries and veins. Change in BP could occur. If there is a consistent increase or decrease in HR or BP the infusion will be stopped and the veterinarian contacted for treatment options.

Phenylephrine: irregular heart rate, respiratory changes, allergic rash. HR, BP and body temp are measured throughout the experiment. If there is a consistent increase or decrease in HR or BP the infusion will be stopped and the veterinarian contacted for treatment options. At this low dose, we do not anticipate behavior.

Acetylcholine: an endothelial cell dilator and thus a vasodilator of peripheral arteries and veins; at the maximal dose used, we do not anticipate severe hypotension or bradycardia; however, if the latter are pronounced or mean arterial BP drops below 40 mm Hg the infusion will be stopped and the veterinarian contacted for treatment options

Serotonin: at this low dose, we do anticipate any change in behavior or long term physiological effects.

Octafluoropropane gas-filled albumin microbubble suspension: No expected adverse effects.

Betamethasone: clumsiness; dizziness; facial flushing; general body discomfort; headache; increased appetite; lightheadedness; nausea; nervousness; pain, swelling, or redness at the injection site; sleeplessness; upset stomach. To reduce possible side effects, animals will be monitored for depreciated appetite or abnormal behavior. If noted discomfort or change in appetite, Pepto Bismol will be given along with more palatable food rations during the 3 day injection period.

ACTH (Cortrosyn): no, or minor, side effects; possibly redness or swelling at the injection site. Rarely, allergic reactions (rash; hives; difficulty breathing; tightness in the chest; swelling of the mouth, face, lips, or tongue); blurred vision; dizziness; headache; irregular heartbeat; severe swelling can occur. During the infusion/injections, all animals will be monitored for BP, HR, respiration and mucosal color. The attending veterinarian and CompMed staff will be notified if any noted severe side effects are observed.

Pepto-Bismol is used for treating heartburn, upset stomach, indigestion, nausea caused by betamethasone treatment.

Letrozole: Administration of Letrozole alone (i.e. without concomitant administration of estradiol) lowers estrogen levels by >95%. When Letrozole treatment is initiated on day 100 and estrogen suppressed, approximately 10% of the baboons abort without any complications (vaginal bleeding visible); the products of conception may or may not be visible in the cage. In this case, the study is terminated, the animal watched closely over the next few days to ensure that bleeding has stopped, appetite is not depreciated and behavior is normal. In another subset of animals (approximately 10%), there is a sudden onset of seizures approximately 25-30 days into the Letrozole treatment. Animals are typically found lying down (comatose) in their cage early in the morning suggesting that seizure(s) most likely occurred overnight or very early that morning. In animals that have seized, we believe it is important to intervene at time of discovery since it is our impression that the longer the animal is left comatose, the more difficult it is to revive the animal. The following protocol seems most relevant to implement and to have been a success in the past:

- Animals which are stuperous (unsteady on their feet but conscious) will immediately be given oral juice/sugar treatment in form of frozen juice or piece of orange or candy to elevate blood glucose levels. If animal is non responsive or progresses to seizing or unconscious state the following will be implemented:
- The animal is removed from its cage and taken to the treatment room. If light sedation is required for safe transport, a small dose (5 mg/kg BW-IM) of ketamine will be administered.
- Blood gas (pO_2, $pC0_2$, pH etc) and glucose will be determined using I-Stat analyzer (results in 2 mins).
- Animal will be placed on O_2 at 2L/min via cone mask and body temperature recorded and maintained with BEAR hugger and warm IV fluids.
- A catheter will be placed in an antecubital or saphenous vein and if blood glucose levels are below 50 mg/100 ml, a 5 ml bolus of 50% Dextrose in Lactated Ringers (1:1) will be delivered over a 5 min period, followed by a 5% Dextrose drip until the animal responds (glucose normalized). Adjustments to normalize pH (e.g. sodium bicarbonate) may also be required.
- Once responsive, the animal is returned to its cage and monitored throughout the day.
- The animal will be removed from the protocol and will most likely abort. If the latter does not occur, the animal will be permitted to go to term and the fetus delivered by cesarean section at the end of treatment.

Finally I want to reiterate that of the 10% that do seize, approximately 35% succumb. It is our impression that these are most likely the animals that exhibited a seizure during the night. We have identified an apparent window between 120-140 days gestation when the seizures are most likely. We (PI staff) have been evaluating changes in glucose levels and blood gases as a way to determine if the seizures will occur. Comp Med personnel, as well as my staff, are aware of this possible linkage and are attempting to resolve this problem. We do want to point out that regardless of when an animal seized, we still will employ the protocol outlined above (i.e. response to question 15a). In addition; decreased appetite can be seen during late treatment with Letrozole. Animal's gums can become swollen making hard biscuit consumption difficult. In this case, affected animals are given biscuits softened soaked in Ensure or Boost to assist in palatability and ensure necessary nourishment. If an animal continues to exhibit inappetence despite this treatment or experiences weight loss, the veterinarian will be consulted.
Estradiol-17β: IV bolus. No adverse effects anticipated.
Estradiol-17β-3 benzoate: SC injections; no adverse side effects anticipated; irritation of skin at the injection site could occur but has not been noted in previous studies
Blood sampling: A potential problem is increased ketamine tolerance, anemia/low hematocrit and sensitivity at injection site. Ketamine tolerance will be managed by using the lowest dose possible for the procedure. We will monitor hematocrit by taking a blood gas reading bi-weekly from animals on study. Vitamin supplements will be given. If prolonged anemia is seen, the Veterinarian will be consulted. To reduce sensitivity at the injection site, when possible the animal will be injected at different sites on the rump or large leg muscle area. In addition to ensure that animals health is not compromised, blood draw will not exceed 10% of the circulating blood volume or 8ml/kg/month. Also note that total blood volume collected from each animal will not exceed 8mL/kg/month

14b. Describe the clinical parameters that will be monitored to indicate adverse effects, pain, and/or distress to animals. The parameters should be specific to the species and to the procedure(s). Include the frequency of monitoring throughout the study.

Animals in the baboon colony are checked by PI staff and twice daily by CompMed staff. All staff will determine if each animal is eating, urinating, passing stool, and demonstrating the repertoire of behaviors normal for the individual animal. Immediately after surgery and during the postoperative period as defined by IACUC policy, animals will be observed daily by PI staff, with these observations recorded on postoperative evaluation sheets which become part of the animals' permanent records. Postoperative evaluation will include specific assessment of pain; failure to eat or decreased appetite, drink, urination or fecal output, change in normal repertoire of behaviors, may indicate pain. Lethargy and guarding of the incision site(s) may also indicate pain. If any of the above is seen during the postoperative monitoring period, the Veterinarian will be notified.

14c. What conditions and/or complications would lead to removal of an animal from the study (*i.e.an early endpoint*)?

Animals will be considered for euthanasia as described in the IACUC protocol entitled "Guidelines for Early Removal Criteria and the Use of Death as an Experimental Endpoint". In addition, the Veterinarian may remove an animal from a protocol if a significant health problem is identified.

G. ADMINISTRATION OF ANESTHESIA, THERAPEUTICS AND EXPERIMENTAL AGENTS

15. Indicate the sedatives/tranquilizers, anesthetics, analgesics, antibiotics, and other relief agents that will be administered. If no anesthetics, analgesics, or other pain relief methods will be used, please provide a strong justification for withholding analgesic agents in Question 15a below. The withholding of analgesic agents must be based upon cited scientific fact or provided experimental data. **NOTE: Some anesthetics and analgesics are controlled substances and require Virginia Board of Pharmacy and DEA licenses for purchase and use.** ADD ADDITIONAL ROWS AS NEEDED.

	Dose (mg/kg)	Route	Frequency
Sedatives/Tranquilizers			
Anesthetics – General			
ketamine-HCl	10-15 mg/kg	IM	Chemical restraint for all protocol blood sampling (1-4 days a week), sedation for IVGTT, ultrasound exam, or preoperatively
ketamine-HCl in 250 ml 0.9% Sodium Chloride IV bag	500mg (2.7ml/min/90)	IV	Chemical restraint for IVGTT, once every 6 months
Isoflurane gas	MAC% is average ~2% for maintenance	Inhaled	At surgery, fetal injection, and during amniocentesis for isolation of amniotic fluid) for determination of fetal sex.
Anesthetics – Local			

Analgesics			Frequency	Length of administration
Flunixin meglumine (Banamine)	2mg/kg	IM	At surgery and minimally 8h following	Surgery + 2 days BID
Antibiotics				
Miscellaneous				
Terbutaline	Recommended dose: 0 25mg to be repeated 30minutes if no clinical change occurs	IV	PRN under veterinarian consult	PRN
Intravenous fluids: 0.9% sodium chloride or similar solution for IV administration	approximately 10 ml/kg body weight per hour during surgery	IV	one dose intraoperative	one dose intraoperative
Iron Dextran	10 mg/kg, IM	IM	At completion of surgery, then PRN not to exceed Q 7days	

15a. **JUSTIFICATION FOR WITHHOLDING ANALGESIC AGENTS**

Analgesics will not be withheld.

16. Will agents other than anesthetics or analgesics (*i.e. drugs, reagents, cells, etc*) be administered?

X YES (*Complete **Question 17** for each agent. Add additional sections as needed.*) _____ NO (*Skip to **Question 18***)

17. Agent: Letrozole Agent vehicle: Sesame oil (sterile)

Route/site: SQ to the mother Volume per administration: 0.2 to 1.0 ml

Frequency of administration: Daily on days 100 to 165-175 of gestation

Expected side effects and/or changes in the animal's behavior: Depreciated appetite. Although the drug itself does not elicit any side effects, the fact that the consequence of drug therapy is a decrease in estrogen production/levels by >95%, we observe in 15%-20% of pregnancies premature delivery and/or maternal seizures. In instances where a mild seizure has occurred but animal has not become comatose, we stop drug treatment for 24-48 hours. Drug treatment can then be re-initiated without further development of any problems. See section 14a.

Agent: Estradiol 17β- 3 benzoate Agent vehicle: Sesame oil (sterile)

Route/site: SC to the mother Volume per administration: 0.2 to 1.0 ml

Frequency of administration: Administered daily on days 100-165-175 of gestation in conjunction with Letrozole to restore estrogen production. Also administered daily on days 25-59 of gestation and on day 54 of gestation in otherwise untreated baboons to prematurely elevate estrogen levels in early gestation.

Expected side effects and/or changes in the animal's behavior: None anticipated

Agent: Estradiol 17β Agent vehicle: Saline/5% ethanol

Route/site: Maternal saphenous IV Volume per administration: < 0.2 ml

Frequency of administration: Once as a bolus to rapidly increase estrogen levels (e.g. 2h and 6h) on day 54 of gestation in untreated animals.

Expected side effects and/or changes in the animal's behavior: None are anticipated

Agent: Serotonin (5-HT) Agent vehicle: 0.9% NaCl

Route/site: IV Volume per administration: 4 µg/kg BW/min/0.3 ml saline and then 8 µg/kg BW/min/0.3 ml saline (each for 20-30 min, i.e. in 'step-up' fashion)

Frequency of administration: Four (4) times over the life span of the animal starting at 8 months of age

Expected side effects and/or changes in the animal's behavior: No expected adverse effects. Any observed change in behavior will be addressed with Veterinarian consult

Agent: Octafluoropropane gas-filled microbubble suspension Agent vehicle: 0.9%saline

Route/site: IV Volume per administration: 0.1ml/min

Frequency of administration: At CEU Doppler study. Total of 16 administrations over the life span of the animal. Not to exceed once a month.

Expected side effects and/or changes in the animal's behavior: None anticipated

Agent: Phenylephrine Agent vehicle: 0.9%saline

Route/site: IV Volume per administration: 1 µg/kg BW/min/0.3 ml saline and then 5 µg/kg BW/min/0.3 ml saline (each for 20-30 min. i.e. in 'step-up' fashion)

Frequency of administration: 4 times over the life span of the animal. Not to exceed once a month.

Expected side effects and/or changes in the animal's behavior: At this low dose, we do not anticipate changes in behavior or physiological function. HR, BP and body temp are measured throughout the experiment. If there is a consistent increase or unlikely decrease in HR or BP the infusion will be stopped and the veterinarian contacted for treatment options.

Agent: Sodium Nitroprusside Agent vehicle: 0.9% saline

Route/site: IV Volume per administration: 1 µg/kg BW/min/0.3 ml saline and then 3 µg/kg BW/min/0.3 ml saline (each for 20-30 min. i.e. in 'step-up' fashion)

Frequency of administration: 4 times over the life span of the animal. Not to exceed once a month.

Expected side effects and/or changes in the animal's behavior: At this low dose, we do not anticipate changes in behavior or physiological function. HR, BP and body temp are measured throughout the experiment. If there is a consistent decrease or unlikely increase in HR or BP the infusion will be stopped and the veterinarian contacted for treatment options.

Agent: Acetylcholine Agent vehicle: 0.9% saline

Route/site: IV Volume per administration: 2 µg/kg BW/min/0.3 ml saline and then 4 µg/kg BW/min/0.3 ml saline (each for 20-30 min. i.e. in 'step-up' fashion)

Frequency of administration: 4 times over the life span of the animal. Not to exceed once a month.

Expected side effects and/or changes in the animal's behavior: At this low dose, we do not anticipate changes in behavior or physiological function. HR, BP and body temp are measured throughout the experiment. If there is a consistent decrease or unlikely increase in HR or BP the infusion will be stopped and the veterinarian contacted for treatment options.

Agent: ACTH (Cortrosyn) Agent vehicle: 0.9% saline

Route/site: IV Volume per administration: 1µg and 10 µg (1-5ml of vehicle)

Frequency of administration: Two injections during suspension study

Expected side effects and/or changes in the animal's behavior: No, or minor side effects, possibly redness or swelling at the injection site. Rarely allergic reaction, blurred vision, irregular heartbeat. If there is a consistent increase or decrease in HR or BP the infusion will be stopped and the veterinarian contacted for treatment options.

NOTE: Your signature on page 4 certifies that all drugs used on animals before, during, or after an experimental or surgical procedure will be obtained from legal sources, will be pharmaceutical-grade, unless otherwise approved, and will be disposed of properly when out-of-date or no longer needed. **All controlled substances MUST be kept in a double-locked compartment, and records documenting each use of a controlled substance MUST be maintained.**

H. SPECIES SELECTION AND ORDERING:

18. Please indicate the species and number of animals requested:

Species (Common Name & Strain)	Total Number Requested for a 3 Year Period	Avg. # to be Maintained in Animal Facility	Max. # to be Maintained in Animal Facility
Baboon (*Papio anubis/cynocephalus*)	140 (5 males, 84 adult females and 51 juveniles)	35	50

If the project involves NON-HUMAN PRIMATES,
Complete Attachment B: NON-HUMAN PRIMATE ENHANCEMENT PROCEDURE.

19. Will animals be ordered through the Division of Comparative Medicine?

X YES _____ NO (***Identify source and provide the rationale/justification***)

20. Will the animals require special housing (e.g., specific bedding requirements, isolator cages, special feed or handling, etc?)

_____ YES (***Describe all special requirements***) X NO

21. Does this study involve the use of animals that will be maintained as a colony over a long period of time? (**Colony is defined as "breeding or holding of animals for reuse in other experiments."**)

X YES (*Complete **Questions 21a and 21b***) _____ NO (*Skip to Question 22*)

21a. List the number of new animals you are planning to purchase for the colony: 3-5

21b. List the number of animals you are planning to use from an existing colony: 40

I. PERSONNEL TRAINING:

22.	In Section 1, list the name of each person involved with the project, along with the species to be used, the person's years of experience with that species, and the person's training information. In Section 2, continuing with the column from Section 1, note each person's functional role for each species listed. **ADD COLUMNS OR PAGES AS NEEDED.**

SECTION 1: PERSONNEL INFORMATION						
NAME:	Gerald Pepe	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
(Species / Experience)	**Species / Exp**	**Species / Exp**	**Species / Exp**	**Species / Exp**	**Species / Exp**	**Species / Exp**
Species used in project / Years of Experience with each species listed	P anubis/ 36	P anubis/15	P. anubis/12	P Anubis/1	P. anubis/2	P. Anubis/5
	/	/	/	/	/	/
	/	/	/	/	/	/
Occupational Health and Safety (OHSP) Training Certificate Number	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Occupational Health and Safety Risk/Health Assessment Date (Month / Year)	7/24/00	7/24/00	3/8/04	1/12/12	9/10/01	5/9/11
LATA Training by species	[redacted] Nonhuman primate	[redacted] Nonhuman primate	[redacted] Nonhuman primate	[redacted] Nonhuman primate	[redacted] Nonhuman primate	[redacted] Nonhuman primate
SECTION 2: FUNCTIONAL ROLE FOR EACH SPECIES LISTED						
Supervision	X		X			
Care and Handing	X	X	X		X	
Anesthesia	X		X			
Surgery	X	X	X	X	X	X
Post-Surgical Care			X		X	
Monitoring	X	X	X		X	
Euthanasia	X		X			

SECTION 1: PERSONNEL INFORMATION						
NAME:	[redacted]					
(Species / Experience)	Species / Exp	Species / Exp				
Species used in project / Years of Experience with each species listed	P anubis/ 1					
	/					
	/					
Occupational Health and Safety (OHSP) Training Certificate Number:	[redacted]					
Occupational Health and Safety Risk/Health Assessment Date (Month / Year)	8/4/11					
LATA Training by species:	[redacted] Nonhuman primate					
SECTION 2: FUNCTIONAL ROLE FOR EACH SPECIES LISTED						
Supervision						
Care and Handing						
Anesthesia						
Surgery	X					
Post-Surgical Care						
Monitoring						
Euthanasia						

22a. Please provide information regarding the degree of training and procedural experience for <u>each</u> individual listed in Questions 22.

Dr. Pepe has performed surgeries on baboons for more than 30 years. His team works closely with him to provide for collection of tissues during surgery. [redacted] has a wealth of experience working with these animals and is now an acknowledged expert in intubation/surgical preparation and performance of surgical procedures outlined in this protocol as well providing surgical assistance. Moreover, [redacted] has been trained by Dr. Pepe and has been performing surgeries (cesarean section) without direct assistance of Dr. Pepe. Although Dr. Pepe has not scrubbed in he is available and now will routinely be on site to assist in collection of fetal tissues. [redacted] has had significant experience assisting Dr. Pepe in the conduct of the surgical experimentation and to collect tissue samples. [redacted] has had over 10 years of experience with surgery in nonhuman primates and is available to assist should [redacted] or others be sick or unavailable. [redacted] did her postdoctoral training with the laboratory group at the University of Maryland and more recently with Dr. Pepe and thus has several years experience working with the nonhuman primate baboon model. [redacted], now an Assistant Professor, will be assisting in collection and performing biochemical analyses of baboon fetal tissues, assisting [redacted] with conduct and analysis of the iv glucose tolerance tests and working with [redacted] and [redacted] with conduct and analysis of ultrasound studies of uteroplacental/fetal blood flow as well as brachial flow and microvascular flow in neonatal and adolescent baboons. [redacted] is the world's authority on use of ultrasound for and determination and analysis of uteroplacental/fetal blood flow in/on pregnancy/pregnancy complications. [redacted] will conduct these analyses in untreated and estrogen treated/deprived baboons and train [redacted] and [redacted] in this regard. [redacted] is a pediatric cardiologist who works alongside [redacted] and is an acknowledged expert in performance and analysis of

fetal cardiac function as well as brachial and microvascular flow and will perform the contrast enhanced ultrasonography analyses of brachial and microvessel flow in neonatal and adolescent baboons. These studies cannot be performed without her.

22b List any person that will require supplemental training from the Division of Comparative Medicine and describe the desired training.

None required

J. ANIMAL USE PROCEDURES (EXCEPT SURGICAL PROCEDURES):

ALL SURGICAL PROCEDURES MUST BE DETAILED IN *ATTACHMENT E, ANIMAL SURGICAL PROCEDURES*

23 Will cells, tissues and organs be collected?

X YES (*Complete all applicable procedures below)* _____ NO (*Skip to **Question 24***)

23a **Blood sampling**

Technique: **[1] Pregnancy studies**: Blood samples (maternal) are collected at 1-5 day intervals between days 25-60, 100-110 or 100-175 of gestation (term = d184). Briefly, animals are restrained, injected with ketamine-HCl (10 mg/kg) and samples (3-5 ml) obtained from a saphenous or antecubital vein using a 21 gauge needle. **[2] Neonate-Adolescent studies**: Blood samples are obtained once every two weeks from neonates and prepubertal adolescents. Briefly, baboons are restrained, injected with ketamine HCl (10 mg/kg BW) and a 2 ml (neonates) sample obtained from a peripheral saphenous vein using a 21g needle. Weight is also recorded and monitored in this group. **[3] IVGTT Studies**: At age 12, 24, 30, 36, 42, 48, 54, and 60 months, and pregnant baboons, animals will undergo intravenous glucose tolerance testing (IVGTT). Samples are collected into syringes via catheter at 0, 1, 3, 5, 10, 20, 40, and 60 mins and 0.1 ml examined for blood glucose and the remainder kept on ice and serum subsequently assayed for insulin/C-peptide. **[4]. Terminal Biopsy**: a baseline blood sample is taken once the animal is prepped to determine blood chemistry and for hormone analysis **[5] Pituitary/Adrenal axis in 7+year old EVMS born baboons:** blood samples are taken over a timed experiment. 9-10 3ml samples total. **[6] BAER test**: one sample at start of the test to determine blood chemistry and hormone analysis **[7] CEU / Doppler:** Two samples are taken, start of the experiment and completion of experiment, for blood chemistry and hormone analysis.

Sample site: Saphenous/antecubital/femoral

Volume per sample: Blood draw will not exceed 10% of the circulating blood volume or 10ml/kg/month for IVGTT, ACTH study. At surgery 3-5 ml but not to exceed 10ml/kg/month

Frequency & duration of sampling: 1-5 day intervals during gestation from mother; once every other week from adolescents; at 6 to 12 month intervals for IVGTT; daily during 2 non-consecutive menstrual cycles in adolescents exhibiting regular menstrual cycles

23b Urine/feces sampling:

Method:

Frequency & duration of sampling:

23c. **Collection of tissues:**

Tissues collected: Kidney, liver, lung, gonads, adrenal, pituitary, pancreas, skeletal muscle, visceral and SQ fat, intestine and uterine samples.

When collected (*before or after euthanasia*): After euthanasia in both adult sacrificed animals and fetus following euthanasia at surgery; also in CEU/ Doppler (skeletal muscle only), SQ and visceral fat at cesarean section as outlined.

Disposition of collected tissues: Fixed and/or frozen for experimentation.

24. Will behavioral testing be conducted?

X No behavioral testing will be conducted

____ Yes, behavioral testing will be conducted with significant restraint or noxious stimuli. (*Complete **Attachment C, PROLONGED PHYSICAL RESTRAINT OR STRESS***)

____ Yes, behavioral testing will be conducted without significant restraint or noxious stimuli. (***Describe the procedure below.***)

25. Will a special diet be required?

X YES (*Complete the **Questions 25a-25c***) ____ NO (*Skip to **Question 26***)

25a. Describe the anticipated nutritional deficit or supplementation:
Vitamins with iron

25b. Provide the reason(s) for and treatment of the deficit or supplementation:
To prevent anemia in animals undergoing blood sampling during pregnancy, IVGTT and or during normal menstrual cycles

25c. How often will animals be weighed?
Adolescents are weighed monthly; pregnant animals, before initiation of experimental drug treatments and at least once during the treatment.

How much weight change will be permitted before the animal will be removed from the study? 20%

26. Will indwelling catheters or implants be used?

____ YES (*Complete a section below for each site. Add additional sections as needed.*) X NO (*Skip to **Question 27***)

26a(1) Site: _____

Type & Size: _____

Maintenance: _____ Duration: _____

26a(2) Site: _____

Type & Size: _____

Maintenance: _____ Duration: _____

27. Will tumors be transplanted or induced?

____ YES (*Complete a section below for each site. Add on more sections as needed.*) X NO (*Skip to **Question 28***)

27a(1) Transplant or Induction Site: _____

Anticipated Functional Deficit(s) and Management: _____

Maintenance: _____ Duration: _____

27a(2) Transplant or Induction Site: _____

Anticipated Functional Deficit(s) and Management: _____

Maintenance: _____ Duration: _____

K. ANIMAL CARE:

28. Describe, in detail, the plans for medical care of the animals in the proposed study, and **identify, by name and job classification**, the responsible person(s) on the investigative staff. **NOTE: *Routine observation of the animals and medical intervention is the responsibility of the principal investigator.***

All animals will be observed daily by [redacted], Animal Coordinator/Research Associate, and/or CompMed staff. Medical problems will be reported to the Veterinarian or a member of the CompMed staff. Postoperative monitoring will be performed for each animal after surgery as defined by IACUC policy. The animal's attitude (alert, responsive) is observed as well as the status of the surgical incision(s), food consumption, urine and feces production, and resumption of the animal's normal repertoire of behaviors; pain is also assessed as described. Postoperative observations are recorded on forms approved by CompMed, and these forms become part of the animal's permanent record.

29. Will this study require special observation?

X YES *(Complete **Question 29a**)* _____ NO *(Skip to **Question 30**)*

29a. Frequency of Observation: daily

By whom *(Identify by Name)*:

[redacted] will be primarily responsible for all observations described above. Animals will also be observed to determine if menstruations have occurred. CompMed or trained member of the PI's staff will observe animals in [redacted] absence.

Starting: daily Ending: daily

30. Indicate any special instructions that should be taken for animals found dead (e.g., call Investigator, refrigerate or freeze carcass, disposal, etc.). If you would like to have the Institutional Veterinarian necropsy animals that die unexpectedly, please indicate how you would like the tissues to be handled. Indicate any special instructions that should be observed for animals found dead (*e.g. call investigator, refrigerate or freeze carcass, disposal, etc.*). **If you would like for the Institutional Veterinarian to necropsy animals that die unexpectedly. please indicate how you would like for the tissues to be handled.**

Alert the investigator and for emergency animal care contact [redacted]. If necropsy is performed, collect uterus and adrenals, and pieces of kidney and liver (or others as determined by the Veterinarian) which are placed in fixative (4% paraformaldehyde or phosphate buffered formalin) for subsequent histopathology.

L. DISPOSITION OF ANIMALS:

31. Please indicate the means of animal disposition (**Check all that apply**):

X Euthanasia *(Complete **Questions 33a-33c**)*

_____ Death as an Endpoint *(Complete **Attachment G: DEATH AS AN ENDPOINT**)*

_____ Return to animal colony

X Available for transfer into another IACUC protocol *

X Available for transfer to another research institution*

_____ Available for adoption as a companion

_____ May be culled for tissue sharing

_____ Other (Explain):

* ***Animals that have undergone survival surgery in one IACUC protocol may not be transferred to another survival surgical protocol, unless the request is specifically reviewed and approved by the IACUC. These animals may be transferred to non-surgical or non-survival protocols without IACUC review.***

32. Disposition of Surviving Animals

32a. Will animals survive the protocol/procedures?

X YES *(Complete **Question 32b**)* ____ NO *(Skip to **Question 33**)*

32b. Will animals survive without harm or disability?

X YES *(Skip to **Question 33**)* ____ NO *(Complete **Question 32c**)*

32c. Describe the harm or disability and the plans for management of the disability.

33. Euthanasia

33a. Will the animals be euthanized?

X YES *(Complete **Questions 33b-33d**)* ____ NO *(Skip to **Question 34**)*

33b. Explain why the animals will be euthanized:

The maximal number of multiple survival surgeries has been achieved or the animal has developed problems either protocol related or clinical which compromise further surgical interventions or the experiment is a terminal procedure. We do attempt to relocate these animals and/or employ them as surrogate mothers for developing neonates. In addition, there is need to collect adult tissues (ovaries, adrenal etc) to serve as controls for our fetal and adolescent studies. In a small % of the colony, we are unable to achieve a pregnancy for reasons not clear to us (the animal does not have normal menstrual cycles, is not mensing at all or is not receptive to breeding). In this instance, we request that these few (<5%) animals be transferred to University of Maryland to be used in our study there.

33c. Indicate how the animals will be euthanized:

Euthanasia agent/procedure: Pentobarbital Solution (390mg/ml)

Dose: 1 ml/4.5 kg body weight Route: IV

33d. Per the *AVMA (American Veterinary Medical Association) Guidelines on Euthanasia*, June 2007 (formerly the *2000 Report of the AVMA Panel on Euthanasia*), most physical methods of euthanasia, when done appropriately, are "conditionally acceptable," meaning that the nature of the techniques may not consistently produce humane death or they present a greater potential for operator error or safety hazards. In those situations where physical methods may be the most appropriate method for euthanasia and rapid relief of pain and suffering, extreme care and caution must be exercised, and personnel performing physical methods of euthanasia must be well trained and monitored for each type of physical technique. **If a physical method, such as decapitation or cervical dislocation, will be used as the primary means of euthanasia, please provide scientific justification.**

N/A

M. ANIMALS BROUGHT INTO AND TAKEN OUTSIDE OF THE ANIMAL FACILITY:

34. Will any animals be transferred into the EVMS Animal Facility from another institution?

X YES *(Complete **Questions 34a-34b**)* _____ NO *(Skip to **Question 35**)*

All animals received from other than approved vendors must undergo a quarantine period to allow for evaluation of the health status of the animals prior to their introduction into the colony. They may also require testing and segregation to determine their health status.

THE PRINCIPAL INVESTIGATOR SHOULD DISCUSS THESE ISSUES WITH THE DIVISION OF COMPARATIVE MEDICINE PRIOR TO INITIATING ANIMAL TRANSFER. THE PRINCIPAL INVESTIGATOR IS RESPONSIBLE FOR ALL RELATED CHARGES.

34a. How long will the quarantine or stabilization period last?

Three completed negative TB test results or as determined by the Institutional Veterinarian

34b. How long will the animals be housed at EVMS?

Animals will be housed at EVMS until 1) the quarantine period is completed, 2) the assigned protocols are completed, and 3) reassignment within EVMS, transfer out of EVMS, or euthanasia. Generally, animals complete the assigned protocols within 1 year. Quarantine and preparation for disposition will add approximately 6 months. Unforeseen problems which disrupt the group may require additional months for stabilization of menstrual cycles before experiments can begin or continue.

35. Will the animals be taken out of the Central Animal Facility (*i.e. CompMed, 4th floor*) for any reason (*i.e. manipulation, surgery, temporary housing, etc.*)?

_____ YES *(Complete **Questions 35a-35c**)* X NO *(Skip to **Question 36**)*

35a. To what building(s) and room(s) will the animals be taken? *(Indicate what procedure(s) will be performed in each specific location.)*

35b. How will the animals be transported? (*Be specific. Include all safety precautions for animals and personnel*.)

35c. How often will the animals be taken to the location(s) listed above, and for what duration of time per incident?

36. Will the animals be used or housed in locations outside of the Central Animal Facility (*i.e. CompMed, [redacted] floor*) for periods greater than 12 hours?

_____ YES *(Complete **Questions 36a-36c**)** X NO

***The location must be certified as a satellite-care facility and undergo semi-annual inspection by the IACUC.**

37a. In what building(s) and room(s) will the animals be used or housed?

37b. Describe the animal husbandry to be performed, and identify, by name, the person(s) who will provide husbandry.

37c. How long will the animals be used or housed in the satellite-care facility?

Eastern Virginia Medical School
Institutional Animal Care and Use Committee

Attachment B: Nonhuman Primate Enhancement Procedure

Project Title: Regulation of Fetal-Placental Development in the Primate

1. Paired housing: Nonhuman primates used under this protocol can be housed in the same primary enclosure with one or more compatible primates.

   X YES (*Skip to Question 2*) _____ NO (*Complete Question 1a*)

   1a. Justify why the animal must be singly housed: animals who are singled housed have demonstrated an inability to pair house. Injury to self or other animals, negative behavioral issues or consistent weight loss is also cause for single housing.

   The PI supports social housing of research primates. However, housing must allow PI/PI staff access to individual animals at all times for blood sampling, injection, and accurate postoperative evaluation.

2. Nonhuman primates used under this protocol will be provided with a variety of devices as described in the EVMS Primate Enhancement Program (this can be provided to you by the Office of Research or the Division of Comparative Medicine (CompMed) upon request).

   X YES _____ NO (*justify in the space below*):

Eastern Virginia Medical School
Institutional Animal Care and Use Committee

Attachment D: Use of Hazardous Agents

Project Title: Regulation of Fetal-Placental Development in the Primate

This form applies to IACUC protocols using materials such as radioisotopes, infectious agents, carcinogens, biohazards, mutagens, teratogens, abortifacients, acutely toxic chemicals, or other potentially hazardous chemicals. Information provided in this form will enable assessment of risk to individuals handling or caring for animals, as well as risk to other animals. **If you need assistance answering any of the questions, please contact the Environmental Health and Safety Office at (757) [redacted].**

The Division of Comparative Medicine (CompMed) must be notified in writing at least one week prior to the use of hazardous agents in animals. The use of hazardous agents in animals is not permitted until approval has been granted by the CompMed Facility Manager. Please reference the CompMed SOP entitled, *Appropriate Notification, Signage and Protocol for Working with Hazardous Agents in Animal Holding Rooms and Procedure Rooms.*

YOU MUST COMPLETE A SEPARATE FORM FOR EACH HAZARDOUS AGENT USED.

1. Please indicate the type of hazardous agent being used. Check all that apply. For microorganisms, please list the Risk Group or Biosafety Level. For chemicals, please list the CAS # and the LD50 from the Material Safety Data Sheet (MSDS).

____ Radioactive material (Isotope: _____)

____ Infectious agent (Risk Group or Biosafety Level: _____)

____ Acutely toxic chemical (CAS #: _____, LD50: _____)

____ Known or suspected human carcinogen (Chemical Name: _____)

__X__ Known or suspected human mutagen/teratogen (Chemical Name: Letrozole_____)

____ Recombinant DNA/RNA

____ Tissue sample (Type: _____)

____ Other (*Describe below*):

2. Please provide specific information about the agent:

Complete name *(Include strain for microorganisms)*: Letrozole

Dose and frequency of administration: Drug is prepared at a concentration of 2 mg/ml sesame oil; animals injected daily with 0.115 mg letrozole/kg BW/day (sesame oil volume ranges from 0.2 to 1.0 ml)

Concentration: 2 mg/ml sesame oil

Route: SC Duration of exposure: 10-60 days (determined by study)

How long will the animal be maintained after administration? Up to 3 years

Animal species: *Papio anubis* Estimated animal weight: 14-18kg

3. Is the agent excreted or shed by the animal?

____ YES *(Indicate the type of excreta and estimated quantity per day)*

X NO

4. Are there documented human risks from exposure to the agent? (Risks may be determined from the MSDS or from references found in the Biosafety Manual.)

X YES *(Complete Questions 4a-4e)* ____ NO *(Skip to Question 5)*

4a. Indicate the route(s) of human exposure:

X Inhalation
X Ingestion
____ Other (describe below):
X Contact
____ Parenteral

4b. Describe the risks associated with the agent (mutagen, teratogen, etc.):

Agent is known to suppress estrogen production in females .Letrozole is an oral, anti-estrogen drug that is used for treating postmenopausal women with breast cancer.

4c. Indicate the acutely toxic/infectious dose in humans (in mg/kg or number of organisms):

The standard dose of Letrozole used in women is 2.5 mg/day. We will be administering a maximum of 2 mg/day and do not anticipate any acute side-effects of the drug per se.

Only PI staff is exposed to the agent

4d. Describe any genetic changes to the organism and their suspected effects:

N/A

4e. Describe the symptoms of exposure: Exposure would have to be long term and contact would need to be ingested or contact with an open wound. Basic exposure symptoms would be irritation or skin rash.

4f. Describe the first aid methods to be taken in the case of exposure:

Flush the area with water

4g. Indicate all personal protection required:

X	Lab coat/dedicated clothing	____	Apron
X	Gloves	____	Face shield
X	Goggles	____	Respirator
X	Other (describe below): Mask		

5. Are there risks to other animals in the room or in the animal facility?

____ YES *(Complete Questions 5a-5d)* X NO *(Skip to Question 6)*

5a. Describe the risk to other animals:

5b. Indicate the route of animal exposure:

5c. describe all methods that will be used to contain the risk factor:

5d. Are special animal care requirements necessary?

____ YES *(Describe below)* ____ NO

6. Are special waste or carcass disposal requirements necessary?

____ YES *(Describe below)* X NO

7. Briefly describe the procedure(s) for administration of the agent and how the agent is metabolized and/or excreted. Include how long the agent is expected to remain in/be shed from the animal, the concentration of agent in any specific organs, etc.

7a. Please indicate whether or not the laboratory personnel and CompMed staff have been informed of these hazards and have received training on proper handling techniques.

	Laboratory Personnel		**CompMed Staff**
X	Yes	X	Yes
____	No	____	No

If not, please contact the Environmental Health and Safety Office to schedule the proper training.

8. Please provide any other relevant information regarding the agent (e.g., unique containment recommendations): This agent is injected SC by PI staff and is not handled by Comp Med staff.

Eastern Virginia Medical School
Institutional Animal Care and Use Committee

Attachment E: Animal Surgery

Project Title: Regulation of Fetal-Placental Development in the Primate

A. PRE-OPERATIVE PROCEDURE

All animal activity proposals involving surgery must provide specific details of pre- through post-procedural care and relief of pain and distress.

1. List by name the person(s) who will be responsible for evaluating the health status of the animals?

   Attending and or Clinical Institutional Veterinarian
   Gerald J. Pepe, Ph.D.
   [redacted]

2. Will food be withheld?

   X YES *(Please explain below and indicate how long food will be withheld)* ____ NO

   Food will be removed late afternoon before surgical procedures scheduled for the following morning. Morning chow will be withheld until after surgery.

3. List all pre-operative anesthetics/analgesics to be used:

   Chemical restraint will be achieved with ketamine HCl (10-15 mg/kg).

4. Describe briefly how animals will be prepared for surgery:

   Surgery will be performed aseptically. Chemical restraint will be accomplished with ketamine. The animal's abdomen, lower legs, and a portion of the back skin (for grounding cautery unit) and forearm will be shaved. Venous access will be established via brachial vein for administration of IV fluids (Lactated Ringers or 0.09% Sodium Chloride). The animal will be intubated, and isoflurane anesthesia will be established. Monitoring of blood oxygen saturation, heart rate, and blood pressure will be initiated. The animal's abdomen will be prepared for surgery by passing iodine-soaked gauze sponges in a rotating pattern, alternating with isopropyl alcohol. This will be done a minimum of 3 times to assure that the skin is as disinfected as possible. The animal will be covered with a sterile surgical drape to establish a sterile field around the abdomen. A catheter is inserted into a saphenous vein and fluids administered to keep the line patent for blood sampling. An initial blood sample obtained from the maternal saphenous vein is analyzed for blood chemistries (e.g. pH, pCO_2, pO_2, HCO_3, oxygen saturation; glucose, Na, K, Cl, etc.) using an I-Stat system. The surgery is a sterile procedure.

B. ANESTHETIC PROCEDURE

5. Will animals be anesthetized?

   X YES *(Complete Questions 6-8)* _____ NO *(Explain below, then skip to Section C)*

6. Who will administer the anesthesia?

   A trained member of the CompMed staff will be primarily responsible for anesthesia. When a CompMed staff member is not available, [redacted] will administer anesthesia.

7. What anesthetic will be used (name and dosage) and how will it be administered? Who will keep records?

   Anesthesia will be achieved with isoflurane gas vaporized with a MAC of 1-2% in 100% oxygen (inhaled). Isoflurane is provided by CompMed, and CompMed will keep anesthesia records. Blood chemistry will be determined using I-Stat instrumentation (pH, pCO_2, pO_2, saturation, Hg, HCO_3, base deficit, etc) which is the property of Dr. Pepe. All records will be available to Comparative Medicine as needed.

8. Explain how anesthetic recovery will be monitored and indicate the person(s) responsible for monitoring the recovery.

The animal will remain on the heated OR table until returned to her cage. The animal will be extubated in the OR when swallowing reflex is observed. She will be returned to her cage when both blinking and swallowing reflexes are observed. Monitoring will be documented at least every 10-15 minutes until the animal is alert and able to maintain a sitting position (Stage 2). Monitoring will be conducted in accordance with IACUC policy and will change if IACUC policy changes. The anesthetist is responsible for monitoring immediate postoperative recovery and documenting recovery using a form developed in consultation with CompMed. The anesthetist will consult with a member of the CompMed staff or the Institutional Veterinarian if problems arise.

C. POST-OPERATIVE PROCEDURE:

Investigators should refer to the EVMS Post-Operative Care Guidelines when developing post-operative procedures.

"Appropriate post-operative records must be maintained in accordance with professionally accepted veterinary procedures regardless of the location of the animal. The attending veterinarian retains the authority to change post-operative care as necessary to ensure the comfort of the animal." AWA, Section 13 and 9 CFR, April 14, 1997.

9. Who will monitor post-operative care on a daily basis?

[redacted] (Animal Coordinator), the PI, or a trained member of the PI staff will monitor recovery according to IACUC policy and will consult with a member of the CompMed staff or the Institutional Veterinarian if the animal appears to be in pain or if complications occur.

10. Who will keep the post-operative record and where will the records be maintained?

Recovery will be documented on forms developed in consultation with the Institutional Veterinarian. These forms will be added to the animal's permanent record maintained by CompMed in the [redacted] floor animal facility.

11. Will post-operative analgesics be administered?

X YES _____ NO (*Explain below, then skip to Section D*):

12. Provide the following information about post-operative analgesia administration:

Agent: Flunixin meglumine (Banamine)

Dose and Route: 2mg/kg IM Frequency: At surgery and 4-8 h following ,2days BID followed PRN up to 5days

Post-Operative Duration of Care: 7days of monitoring

D. MULTIPLE SURVIVAL SURGERY

All multiple survival surgery must be conducted in accordance with EVMS' Multiple Survival Surgery Policy.

The Animal Welfare Act does not allow for animals to undergo more than one (1) survival surgery unless scientifically justified by the principal investigator, appropriate for the animal's veterinary care, or approved by the USDA administrator. The IACUC must specifically review and approve every additional surgery to be performed on a single animal.

13. Will the animal be subjected to more than one survival surgery?

X YES *(Answer Questions 13a-13b)* _____ NO *(Skip to Question 14)*

13a. Please briefly outline the procedures, explain how the surgeries are related and justify the need for more than one surgery per animal:

The protocol is designed to elucidate the role of estrogen on placental fetal development and function and impact on adrenocortical self-sufficiency in the perinatal period and reproductive function in adulthood. Thus surgeries are related to each other both by development and by estrogen. Thus, we study the animal discrete times in control (no treatments) pregnancy, e.g. days 60, 100, 110, 120, 140 and 160/165 a time

during which there is increased endogenous production of estrogen by the placenta. We then examine in that same animal the effect of removing estrogen (e.g. between day 100 and 170) or giving estrogen earlier (day 25-59). We also need to have a control for our drug manipulations and thus in animals in which estrogen is depleted by treatment with Letrozole, the animal is also studied following treatment with Letrozole and estradiol. Thus, each animal essentially serves as its own control. The major survival surgery performed is a cesarean section.
Animals reared at EVMS from neonate to adulthood, once puberty has been reached and normal menstrual cycles exhibited, will be hemi-ovariectomized.

13b. How many surgeries will each animal experience?

Each animal may undergo up to six (6) major survival surgeries without complications to the animal. While this is the optimal number to achieve statistically valid data, we work closely with the EVMS veterinarian to be sure that animals are healthy and have no untoward medical and/or behavioral complications (adhesions; uterine windows etc.) that would not be compatible with performing another surgery.

14. Have any animals to undergo major survival surgery in this protocol already undergone major survival surgery in any other IACUC protocols?

YES *(Answer Questions 14a-14d)*
*However, they were not used in an unrelated protocol. This protocol is the 3-year continuation of IACUC #09-007

X ____ _____ NO *(Skip to Section E)*

14a Identify all animals that have undergone prior surgical procedures in another protocol:

Please see surgery log submitted to the committee

14b. Identify all of the previous procedure(s) involved:

Please see surgery log submitted to the committee

14c. Identify the IACUC protocol number(s) the previous surgeries were performed under:

09-007. This protocol (IACUC #12-010) is the 3-year continuation of IACUC #09-007.

14d. In accordance with the Animal Welfare Act, the IACUC must specifically review and approve all requests for the use of animals that have undergone previous survival surgery under another IACUC protocol. Please justify the need to reutilize such animals in this surgical protocol:

The animals in this protocol were used in the prior approved IACUC protocol (i.e. research is continuous). The multiple use of (the same) baboons reduces the total number of animals required for conduct of the study and still permit collection of statistically valid data. Thus, we study the role of estrogen in the same baboon (i.e. experiments are interrelated/integrated) during control periods (e.g. on day 60 and on day 160-170 of gestation), on day 60 following treatment with estrogen or on day 160-170 following treatment with aromatase inhibitor or aromatase inhibitor plus estrogen, i.e. one animal rather than 5 animals are studied. Multiple pregnancies also mimics the situation in humans.

E. SURGICAL PROCEDURES:

15. Is the surgical procedure to be addressed on this page a major surgical procedure or a minor surgical procedure (a major surgical procedure is defined as involving penetration of a body cavity and/or resulting in a disability or dysfunction in the animal):

_____ Major surgical procedure X ____ Minor surgical procedure

16. Is this survival surgery?

X ____ YES _____ NO

17. Indicate what number the surgery described on this page is (i.e., only procedure to be performed, 1st surgical procedure, 2nd surgical procedure, etc.) If you are performing the same exact surgical procedure more than once on a single animal, indicate below how many times you will be performing this procedure on the animal (in this case, you will only need to submit one copy of this page for that specific procedure):

The procedure to be performed is an amniocentesis between day 80-95 of gestation. Amniotic fluid is obtained for fetal sex determination. This is performed 6-10 times max on each animal. (some animals will spontaneously deliver resulting in no surgery or abort spontaneously resulting in no surgery. 6 would be the max if each pregnancy ended in a surgery)

18. Indicate the location (provide a specific room number if surgery will be conducted outside of the Comp Med animal facility, what time of day and what day(s) of the week surgery will be performed. Take into consideration the time necessary for care after anesthesia and surgery:

Surgical procedures will be performed in an approved nonhuman primate surgical suite located in the CompMed vivarium between 8 am and 4 pm, Monday-Friday.

19. Describe the entire surgical procedure:

Objective is to determine the sex of the fetus. Briefly, pregnant baboons are sedated with ketamine, and anesthetized with isofiurane using a nose cone. The fetus, fetal head, limbs etc and placenta are localized by ultrasound (not sterilized). A sterile 22 gauge needle (10 ml syringe attached) is then inserted through the abdomen and uterine wall and into the amniotic fluid away from the fetus/placenta and approximately 10 ml of amniotic fluid is removed. Fetal heart rate is monitored before and after the procedure to ensure that the fetus has not been compromised. The animal is returned to its cage and monitored by staff.

E. SURGICAL PROCEDURES:

15. Is the surgical procedure to be addressed on this page a major surgical procedure or a minor surgical procedure (a major surgical procedure is defined as involving penetration of a body cavity and/or resulting in a disability or dysfunction in the animal):

X Major surgical procedure _____ Minor surgical procedure

16. Is this survival surgery?

X YES _____ NO

17. Indicate what number the surgery described on this page is (i.e., only procedure to be performed, 1st surgical procedure, 2nd surgical procedure, etc.) If you are performing the same exact surgical procedure more than once on a single animal, indicate below how many times you will be performing this procedure on the animal (in this case, you will only need to submit one copy of this page for that specific procedure):

The only surgery to be performed in pregnant baboons is a cesarean section. No other surgeries are performed on pregnant animals. Cesarean sections can be performed up to six times on one animal as long as no adverse health changes occur in the animal(inability to sustain pregnancy, repeated failure to become pregnant, unhealthy uterus) This would be determined with PI/Vet consultations and could be cause for early termination.

18. Indicate the location (provide a specific room number if surgery will be conducted outside of the Comp Med animal facility, what time of day and what day(s) of the week surgery will be performed. Take into consideration the time necessary for care after anesthesia and surgery:

Surgical procedures will be performed in an approved nonhuman primate surgical suite located in the CompMed vivarium between 8 am and 4 pm, Monday-Friday

19. Describe the entire surgical procedure:

To determine the role of estrogen on placental-fetal development and impact on function in adulthood. On day varying days of gestation determined by the study group, baboons are briefly restrained in home cage via squeeze mechanism, injected with ketamine (10 -15mg/kg), intubated, anesthetized with isoflurane/oxygen and vitals (e.g. heart rate, blood pressure) monitored by CompMed staff. A 22g catheter is placed in the brachial vein and IV fluids administered (Lactated Ringers Solution/NaCl). A second catheter is placed in the saphenous vein using a 19g catheter 24inches in length and fluids administered. The animal's abdomen/ surgical site is shaved and scrubbed with alcohol and Betadine solution. The animal is draped using sterile technique. Blood samples are obtained from the mother at '0' time, mid procedure and post placental delivery via saphenous catheter. Prior to surgery, a proper plane of anesthesia will be assured by lack of response to a noxious stimulus such as toe pinch. A vertical mid-line incision

is made using a 10blade. Once the abdomen is open, the uterus is externalized and examined for placenta location. Mother's ovaries are examined for evidence of ovulation/corpus luteum. Two to four uterine vein samples are taken (5 ml) using a 23g needle. Fetal crown is localized and a horizontal incision is made in the uterus to remove the fetus. Amniotic fluid is recovered using a syringe. Once the fetus is carefully delivered, umbilical vein and artery samples are taken using a 23g butterfly set (4-8 ml) for hormone, steroid and blood gas analysis. The abdomen and uterus are rinsed with warm saline as needed to prevent drying and to clean the area. The umbilical cord is double clamped to ensure the safety of the mother; the fetus is then euthanized by injecting the umbilical artery with Fatal Plus euthanasia solution. After the fetus expires (no heart beat), the cord is cut, placenta is delivered. Cardiac stick is used as a secondary means of euthanasia. Segments of the placenta and the fetal adrenal, hypothalamus, pituitary gland, lungs, kidneys, liver, skeletal muscle, pancreas, subcutaneous and visceral fat, brain and heart, and gonads are collected, portions fixed in formalin or snap frozen for subsequent immunocytochemical-biochemical/ mRNA determinations. The uterus is cleaned and closed absorbable suture. The uterus is manually messaged to stimulate contractions and shut down bleeding. Once closed, the uterus is rinsed with sterile saline and placed back in the abdomen. The abdomen is then rinsed with sterile saline to remove any blood clots. Prior to closing the abdomen, a small 10-15gram sample of visceral/abdominal fat is ligated and removed for RNA analysis. The abdomen is closed by three layers; the first layer (peritoneum), if present, a second layer (fascia) and if a clear fascia layer is not present then a SQ layer is closed using a continuous stitch. Finally, the skin is closed. Vet-bond adhesive glue is applied to the incision line once skin is closed. Since dissolvable suture material is used, no suture removal is required. The mother is injected with 10mg/kg IronDextran IM for iron supplementation. Flunixin meglumine (Banamine) is given IM during immediate recovery. Isoflurane is shut off and animal is monitored until swallowing is present. Once able, the animal is extubated and continues to be monitored in home cage until fully upright and responsive to stimuli. Immediate post-op monitoring is done by Com Med staff using procedures outlined in the EVMS Post-Operative Care Guidelines Manual and in Attachment E below. PI staff is also present during immediate recovery. In selected experiments, the fetus is delivered live either spontaneously (on day 184) or by cesarean section on day 170 and reared to adulthood.

In some experiments (day 165-175 only), the fetus-neonate is not injected with pentobarbital since it will be reared to adulthood. Live fetuses are cleared of mucous, stimulated to breathe, placed in a warm blanket until returned to the mother for sub-sequent rearing to adulthood. If neonate is not received by the mother for unknown reasons and fails to thrive, the neonate will be hand reared until old enough/strong enough to be introduced to age appropriate conspecies. In some cases, a surrogate mother can be used to nurse the neonate. This is the best cases. Neonate rearing is managed and designed in cooperation with CompMed and the attending Vet.

E. SURGICAL PROCEDURES:

15. Is the surgical procedure to be addressed on this page a major surgical procedure or a minor surgical procedure (a major surgical procedure is defined as involving penetration of a body cavity and/or resulting in a disability or dysfunction in the animal):

X Major surgical procedure _____ Minor surgical procedure

16. Is this survival surgery?

X YES _____ NO

17. Indicate what number the surgery described on this page is (i.e., only procedure to be performed, 1st surgical procedure, 2nd surgical procedure, etc.) If you are performing the same exact surgical procedure more than once on a single animal, indicate below how many times you will be performing this procedure on the animal (in this case, you will only need to submit one copy of this page for that specific procedure):

The 1st survival surgery to be performed in adolescent baboons is removal of one ovary (hemi-ovariectomy) at approximately 96 months of age (i.e. after fertility testing by breeding). These animals, born to mothers untreated or treated *in utero* with Letrozole or Letrozole plus estradiol, will have had no prior major surgeries. *In vivo* response of the remaining ovary to pituitary gonadotropin will be initiated approximately 9 months after hemi-ovariectomy and animals have exhibited at least three consecutive normal menstrual cycles.

18. Indicate the location (provide a specific room number if surgery will be conducted outside of the Comp Med animal facility, what time of day and what day(s) of the week surgery will be performed. Take into consideration the time necessary for care after anesthesia and surgery:

Surgical procedures will be performed in an approved nonhuman primate surgical suite located in the CompMed vivarium between 8 am and 4 pm, Monday-Friday

19. Describe the entire surgical procedure:

Objective is to determine the role of estrogen *in utero* on ovarian development and fertility in adulthood. Juvenile to adult baboons from Experiment II, Groups 5,6, and 7 are briefly restrained in home cage via squeeze mechanism, sedated with ketamine (10-15mg/kg), intubated, anesthetized with isoflurane/oxygen and vitals (e.g. blood pressure) monitored by CompMed staff. A 22g catheter is placed in the brachial vein and IV fluids administered (Lactated Ringers Solution). A baseline blood sample (5ml) is taken using a 21g needle for blood gas analysis and hormone analysis. The animals' abdomen/surgical site is shaved and scrubbed with alcohol and Betadine solution. The animal is draped using sterile technique. Prior to surgery, a proper plane of anesthesia will be assured by lack of response to a noxious stimulus such as toe pinch After making a small abdominal incision a retractor is put in place, lap sponge can be used if needed for a clearer view, ovaries are located. Once the ovulation site is located, the ovarian ligament and vasculature is identified, clamped and cauterized and the ovary removed. Once removed, clamps are carefully removed and ligament/cautery site observed for bleeding. Abdomen is rinsed with sterile saline. Abdominal layers are closed (peritoneum, fascia/SQ and skin). Vet-bond skin adhesive applied to the incision site once closed. Since dissolvable suture material is used, no suture removal is required. Flunixin meglumine (Banamine) is given IM during immediate recovery. Isoflurane is shut off and animal is monitored until swallowing is present. Once able, the animal is extubated and continues to be monitored in home cage until fully upright and responsive to stimuli. Immediate post-op monitoring is done by Com Med staff using procedures outlined in the EVMS Post-Operative Care Guidelines Manual and in Attachment E below. PI staff is also present during immediate recovery.

E. SURGICAL PROCEDURES:

15. Is the surgical procedure to be addressed on this page a major surgical procedure or a minor surgical procedure (a major surgical procedure is defined as involving penetration of a body cavity and/or resulting in a disability or dysfunction in the animal):

_X___ Major surgical procedure _____ Minor surgical procedure

16. Is this survival surgery?

_____ YES _X___ NO

17. Indicate what number the surgery described on this page is (i.e., only procedure to be performed, 1st surgical procedure, 2nd surgical procedure, etc.) If you are performing the same exact surgical procedure more than once on a single animal, indicate below how many times you will be performing this procedure on the animal (in this case, you will only need to submit one copy of this page for that specific procedure):

This is a terminal biopsy surgery. Adult EVMS reared animals will not recover from this procedure.

18. Indicate the location (provide a specific room number if surgery will be conducted outside of the Comp Med animal facility, what time of day and what day(s) of the week surgery will be performed. Take into consideration the time necessary for care after anesthesia and surgery:

Surgical procedures will be performed in an approved nonhuman primate surgical suite located in the CompMed vivarium between 8 am and 4 pm, Monday-Friday

19. Describe the entire surgical procedure:

As a terminal procedure for those now adult EVMS reared baboons that have completed the already approved juvenile study, we propose the following: Baboons are sedated with ketamine (10-15mg/kg im) and prepped/ monitored for the procedure under Comp Med SOP. A baseline blood sample (5ml) is obtained from the saphenous vein using a 21g needle to determine blood chemistries (Na, K, etc), gases (pCO_2, pO_2) and acid/base status and subsequent analysis of estradiol, progesterone and androgens. Heart rate, blood pressure, respiration rate, oxygen saturation and body temp are monitored by Comp Med throughout the procedure; at surgical plane of anesthesia is maintained. A 2-3cm incision is made in the bicep to expose the muscle. A section of the muscle will be removed using either a surgical blade or dissecting scissors. The incision will be secured with a running stitch. A second incision will be made from the xiphoid process to the pubis. A section of abdominal and subcutaneous fat will be excised. Finally, the pancreas is isolated using blunt dissection. One biopsy is taken from the tail region (2.3mm x 85m). The surrounding vessels are clamped immediately to control bleeding. The pancreas is then completely removed for further sampling. As soon as the pancreas is free, Fatal Plus (Pentobarbital Solution) euthanasia solution is administered (1ml/4.5kg/bw) IV followed by a saline flush. Euthanasia is confirmed by the absence of HB and flat ECG. Subsequent administration of FP will follow PRN until death is confirmed.

IACUC Surgery Documentation for Multiple Survival Surgeries

boon #	sx date	sx date	sx date	sx date	sx date	sx date	sx date	sx	live birth	preg	abort
13812	Sep-04	Jun-06	Mar-08	Mar-09	Oct-11			5		6	1
September 2004 post surgical complication: animal's suture line opened and needed reclosing by the veterinarian. Since then a stronger type of suture is used on the baboons											
14584	Dec-05	Oct-07	Nov-08	Sep-09	Jan-11			5	1	8	2
No noted surgical complications											
15801	Jun-08	Apr-09	Mar-10	Feb-11				4	2	7	1
No noted surgical complications											
15761	Oct-07	Jul-08	Apr-09	Mar-10 preg				4	2	7	
No noted surgical complications											
15011	May-07	Jan-08	Feb-09					3	1	4	
No noted surgical complications											
16917	Aug-07	1-Mar	Feb-10					3		3	
No noted surgical complications											
17453	Aug-08	Jun-10						2	2	4	
June 2010 NOT a surgical complication. Animal presented with a prolapsed vagina. No indication this is a result of surgery. Possibly remove from study for this reason.											
18631	Jul-09	Oct-10						2	1	3	
No noted surgical complications											
18660	1-Jan	Sep-10	Feb-12					3		5	2
No noted surgical complications											
18652	3/11[tc]	4/12[tcgue2]						2		3	1
No noted surgical complications											
19045	8/09[tc]	11/10[tc]						2		2	
No noted surgical complications											
28768											
29152											
28741											
26876											
26745											
27320											

EVMS born animals

boon #	sx date	sx date	sx date	sx date	sx date	sx date	sx date	sx	live birth	preg	abort
11790A	Jan-08							1			
G194	Dec-08							1	1	1	
H002	May-10							1		1	1
M35A	Dec-08							1	1	1	
I153	Nov-07							1			
H080	Oct-09							1			
I096	Dec-04							1	1	1	

No noted surgical complications

L059

14884A

M183

M184

N026

O002

O079

Male breeders only

2535

11358

15069

116-12

EVMS
Eastern Virginia Medical School

October 15, 2014

Gerald J. Pepe, Ph.D.
Chair, Department of Physiological Sciences
Eastern Virginia Medical School
[redacted]
Norfolk, Virginia 23507

Dear Dr. Pepe:

The Institutional Animal Care and Use Committee has reviewed your request to amend the protocol entitled, ***Regulation of Fetal-placental Development in the Primate*** **(IACUC #12-010). The following amendments to the protocol have been approved via the facilitated review (FR) process:**

1. **sedation/anesthesia of maternal baboons and baboon offspring using a constant IV infusion of Ketofol (i.e., a mixture of Ketamine and Propofol) instead of Isoflurane and orotracheal intubation. The sedation/anesthesia method will apply to all approved invasive and non-invasive procedures except for cesarean sections.**
2. **permission to perform a dose-response preliminary study in 4 baboon offspring greater than 7 years of age to validate the appropriate dose of Ketofol.**

Although the Division of Comparative Medicine (CompMed) will make every effort to provide housing, husbandry, and veterinary support for your research project, IACUC approval of this amendment request does not guarantee that CompMed will be able to accommodate the work immediately. Available space and resources within the CompMed animal facility are determined by the number of ongoing projects and the animal census at any given time. Please consult with the CompMed management team to confirm availability prior to implementing the approved modification(s).

It is the responsibility of the principal investigator to notify the Committee, in writing, of any deviation from the IACUC-approved protocol. Please note that a change in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian prior to submitting the amendment request to the IACUC for approval.

It is also the responsibility of the principal investigator to provide for regular observation of the health of the research animals and to provide the Division of Comparative Medicine (CompMed) with the name(s) and telephone number(s) of the person(s) who may be contacted and/or take action in the event that the CompMed staff notes an emergency condition with the research animals.

Thank you for your continued cooperation with the Institutional Animal Care and Use Committee.

Sincerely,

[redacted]
Chair
Institutional Animal Care and Use Committee

[redacted]

cc: [redacted]
Attending Veterinarian
Division of Comparative Medicine

[redacted]
Project Manager
Division of Comparative Medicine

[redacted]
Senior Associate Dean for Research
Institutional Official

[redacted] www.evms.edu
P.O. Box [redacted] Norfolk, VA 23501-1980 Phone: 757-[redacted] Fax: 757-[redacted]

EVMS
Eastern Virginia Medical School

October 14, 2014

Gerald J. Pepe, Ph.D.
Professor and Chair

[redacted] Chair
Institutional Animal Care and Use Committee
Eastern Virginia Medical School
Norfolk, VA 23507

Dear [redacted]:

I would like to thank you and the IACUC members for the prompt and thorough review of my October 6, 2014 request to amend my approved IACUC protocol entitled "Regulation of Fetal-Placental Development in the Primate" (#12-010). Hopefully, the following brief narrative and proposed changes address the two interrelated questions/issues of the IACUC Committee as outlined in the October 13, 2014 email from [redacted].

Briefly, over the years we have developed significant experience using intravenous infusion of ketamine alone. Based on the latter and data on "ketofol" in the literature, we anticipate that the "ketofol" doses proposed will be satisfactory. Nevertheless, we do need to prove that and be prepared and describe how animals will be managed should the highest dose of "ketofol" prove inadequate. To address these issues we propose the following. We agree with the Committee and thus request that the preliminary study be performed in four baboons. In the first animal, should the doses of ketofol proposed initially produce the desired effects, we would repeat such in the second animal and 3rd animal. However, should the doses originally outlined not produce the desired effects in this first animal (or be repeated in the second animal), the infusion will be terminated at the 30 minute period regardless and the animal returned to its cage and if necessary injected im with ketamine to ensure safety/produce restraint (10 mg/kg BW). Moreover, in this scenario, for the second (or third) animal, we propose elimination of the lowest dose (0.05 mg ketamine /0.10 mg propofol) and propose starting (time 0) with the 0.1 mg ketamine: 0.20 mg propofol dose; at 10 min, increase that dose to 0.15 mg ketamine: 0.30 mg propofol and at 20 min increase the dose to 0.20 mg ketamine/0.35 mg propofol. If desired effects are achieved we would repeat this dosage regiment in animals 3 and 4. If desired effects are still not achieved, we would need to reevaluate the doses/combination and make a request for change in doses/combination to the IACUC for the 3rd and 4th animals.

Hopefully this answers the 2 questions posed by the Committee. The IACUC's time and effort in reviewing this revised amendment is most appreciated.

Sincerely,

Gerald J. Pepe, Ph.D.

April 2010
Updated February 2011; March 2011; April 2012; August 2013

REQUEST TO AMEND A PREVIOUSLY APPROVED IACUC PROTOCOL

Institutional Animal Care and Use Committee (IACUC)

Eastern Virginia Medical School
Office of Research

IACUC Office
[redacted]

Office: 757-[redacted]
Fax: 757-[redacted]

FOR OFFICE USE ONLY
Date Received: 10/14/14 [redacted] **Review Method:** ____ FCR / X FR / ____ Administrative (*Personnel Changes Only*)
IBC Approval? X Yes / ____ No **IBC Approval Date:** 10-6-14 **Final Approval Date:** 10/15/14

General Information and Instructions:

1. All requested amendments must be approved by the IACUC before they are implemented.
2. Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian before the changes are submitted to the IACUC Office.
3. A request to increase the number of animals assigned to a protocol must be approved by the IACUC before the animals are ordered.
4. Submit the original signed amendment form to the IACUC Office located in [redacted], and e-mail the MSWord version of the form to the IACUC Administrator.
5. *The IACUC reserves the right to require submission of an IACUC "Initial Review Form" based upon the Committee's assessment of the amendment(s) requested herein.*

I. ADMINISTRATIVE INFORMATION

Protocol Number: 12-010	**Protocol Initial Approval Date: 7/30/2012**
Protocol Title: Regulation of Fetal Placental Development in the Primate	
Principal Investigator: Gerald J. Pepe Ph.D.	
Approved USDA Pain Code Level(s) – *not required for personnel additions only*: **B & D**	

II. PERSONNEL

List all personnel to be added to or removed from the protocol. All persons authorized to work on the protocol must have completed the required CITI/LATA and OHSP training modules and must have completed a health/risk assessment with the Occupational Health Department.

Name	Added (+) or Removed (-)	E-mail Address *(additions only)*	CITI/LATA Training Certification Number *(additions only)*	CITI/LATA Training Species Module(s) Completed *(additions only)*	OHSP Training Certification Number *(additions only)*	Occupational Health/ Risk Assessment Date *(additions only)*
1.						
2.						
3.						
Procedure(s) to be performed by personnel addition #1:						
Procedure(s) to be performed by personnel addition #2:						
Procedure(s) to be performed by personnel addition #3:						

☐ **A. CHANGE OF SPONSOR/FUNDING SOURCE**

List the new/approved funding source, the project/grant number, the project/grant title (if different from the IACUC protocol title), and the award period.

☐ **B. CHANGE IN PROJECT SITE(S)**

List all new locations (i.e., building and room number(s)) and indicate what procedure(s) will be performed in each new location.

☐ **C. CHANGE OF SPECIES AND/OR STRAIN**

List the species to be added to or removed from the protocol. Please provide the rationale for any species/strain additions and indicate the USDA Pain Category assignment for each new species/strain. Also, list any anticipated changes in veterinary care and/or husbandry due to the new species/strain.

Species and/or strain	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E

☐ **D. CHANGE IN ANIMAL NUMBERS**

List the number of animals to be added to or removed from the protocol. If animals will be added, please provide the justification for the increase and indicate the number of animals to be assigned to each USDA Pain Category.

Species and/or strain	Year (1, 2 or 3)	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E	TOTAL

Will the animals be ordered through CompMed? _____Yes _____ No ***(Identify the source and provide the rationale/justification below.)***

Will the animals require special housing (e.g., specific bedding requirements, isolator cages, special feed, etc.)
_____ Yes ***(Please specify below.)*** _____ No

USDA Pain Code B – Breeding or holding colony; no animal manipulation.
USDA Pain Code C – Procedures involving no or momentary slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D – Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E – Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

[X] **E. CHANGE IN EXPERIMENTAL PROCEDURES AND/OR INSTRUMENTATION**

Describe, in detail, the proposed change(s). *Please indicate potentially painful/distressing effects on the animals as a result of the proposed change(s). Also, please indicate any expected adverse effects and any changes in monitoring, husbandry, housing, early endpoint, and/or experimental endpoint. Finally, please complete the checklist below.*

In this amendment, we request approval to substitute the method of anesthesia/sedation of maternal baboons and baboon offspring from isoflurane/orotracheal intubation to ketamine:propofol ("ketofol") via constant iv infusion for all approved invasive/non-invasive procedures other than major surgery (i.e. cesarean section). Studies have shown that isoflurane causes extensive vasodilation, significantly lowers MABP and markedly decreases pancreatic Beta cell function and thus insulin release, effects which compromise the research and which were criticized by study section review of our recent R01 NIH grant submission. It is now well established that the combination of ketamine:propofol uniquely provides highly safe and effective sedation/analgesia to permit numerous clinical interventions (e.g. bone marrow aspiration; endometrial biopsy), in patients including those coming to emergency rooms with varied painful injuries (e.g. fracture manipulations) as well as changing of dressings in children with severe burns. Moreover, and very importantly, the combination of ketamine (0.1 mg/kg) and propofol (0.2 mg/kg) reduced the amount of propofol required and thus reduced propofol consumption, preserved hemodynamic stability including cardiopulmonary function and MABP and improved recovery quality. A copy of the first page of several of these articles is appended with the letter of request.

We are also requesting to perform a preliminary study in four baboon offspring >7 years old to validate the dose of ketamine:propofol. Specifically, as outlined in the appended letter and its attachments, we request permission to perform a dose-response study in which MABP, heart rate are monitored during and blood gases/chemistry and levels of glucose, insulin, and cortisol determined in peripheral blood samples (3 ml) as already approved and obtained before and after infusion of ketamine:propofol for 10 minutes at doses of 0.05 mg/kg : 0.10 mg/kg respectively, then at 0.10 mg/kg : 0.20 mg/kg for 10 minutes and then 0.15 : 0.25 mg/kg for the final 10 minute period. We anticipate the dose used in humans (as well as beagle dogs) and provides sedation/analgesia and cardiopulmonary homeostasis (0.1 mg ketamine and 0.2 mg propofol per kg bw/min, iv) will be applicable to the baboon. However, should these doses not produce the desired effects in the first animal (or be repeated in the second animal), the infusion will be terminated at the 30 minute period regardless and the animal returned to its cage and if necessary injected im with ketamine to ensure safety/ produce restraint (10 mg/kg BW). Moreover, in this scenario, for the second (or third) animal, we will eliminate the lowest dose (0.05 mg ketamine /0.10 mg propofol) and start (time 0) with the 0.1 mg ketamine: 0.20 mg propofol dose; at 10 min, increase that to 0.15 mg ketamine: 0.30 mg propofol and at 20 min increase that to 0.20 mg ketamine/0.35 mg propofol. If desired effects are achieved we would repeat this dosage regiment in animals 3 and 4. As there is anticipated animal variability/response, the lower and higher doses will permit us to determine impact of having to provide animals during study a little more or a little less sedative.

Please check all that apply to the proposed change(s). Complete the required IACUC Attachment and submit it along with the amendment form.	
Biohazardous Agents: ____ Yes __X__ No (e.g., recombinant DNA, RNA, all tissue or cell samples, laboratory-induced infection, cultured pathogens)	Complete and submit **Attachment D**
Radioisotopes, *in vivo*: ____ Yes __X__ No	Complete and submit **Attachment D**

USDA Pain Code B – Breeding or holding colony; no animal manipulation.
USDA Pain Code C – Procedures involving no or momentary slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D – Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E – Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

Chemical Agents: ____ **Yes** __X__ **No** (e.g., known or suspected chemical hazards, mutagens, or teratogens)	Complete and submit **Attachment** D
Stress or Prolonged Restraint: ____ **Yes** _X___ **No**	Complete and submit **Attachment** C
Food and/or Water Deprivation: ____ **Yes** _X___ **No**	Complete and submit **Attachment** C
Surgical Procedures: ____ **Yes** _X___ **No** (i.e., single survival, multiple survival, or non-survival)	Complete and submit **Attachment** E
Antibody Production: ____ **Yes** __X__ **No**	Complete and submit **Attachment** F
Toxicity Testing (LD50): ____ **Yes** _X___ **No**	Complete and submit **Attachment** G
Lasers or Penetrating Electromagnetic Radiation: ____ **Yes** __X__ **No**	
Collection of Tissues, Cells, or Organs: ____ **Yes** __X__ **No** (Please explain below.)	
Tumor Transplantation or Induction: ____ **Yes** _X___ **No**	
Special Diet: ____ **Yes** _X___ **No** (Please explain below.)	
Other : (Please specify below.)	

X **F. CHANGE IN ANESTHESIA, THERAPEUTICS, AND/OR EXPERIMENTAL AGENTS**

	Dose (mg/kg)	Route of Administration	Frequency of Administration
Sedatives/Tranquilizers			
Anesthetics - General			
Ketamine	0.05-0.20mg / kg/min	IV in saline	In maternal baboons and baboon offspring during iv glucose tolerance tests (ivGTT) to include muscle biopsy at time 0 and 30 mins after glucose injection; during Doppler studies of brachial flow-mediated dilation before and after vaso-challenge to include muscle biopsy before and after challenge; Doppler study of utero-placental-fetal blood flow before/after serotonin
Propofol (Diprivan)	0.1-0.35 mg/ kg/min	IV in saline	As above
Anesthetics – Local			
Analgesics			

	Length of Administration

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used SCIENTIFIC JUSTIFICATION IS REQUIRED.

EXPERIMENTAL AGENTS:

Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _____

INVESTIGATOR ASSURANCES:

X	*I understand that it is my responsibility as the Principal Investigator to ensure that all personnel who work on this protocol are adequately trained. That training may be provided by me, an experienced member of the investigative staff, the Attending Veterinarian, a qualified Division of Comparative Medicine (CompMed) staff member, or an appropriate outside source. I hereby affirm that each person added to this protocol by way of this amendment request has been adequately trained to perform the procedure(s) indicated above. Any person who has not been adequately trained will be so trained prior to performing the procedure(s).*
X	*No animal involved in this project will be subjected to discomfort, pain, or distress, unless it is unavoidable in the conduct of scientifically valuable research and approval of such has been approved by the IACUC.*
X	*I agree to comply with all federal and institutional policies governing the use of animals used in this project.*

PRINCIPAL INVESTIGATOR:

Printed Name: Gerald J Pepe, Ph.D.

Signature: [signature] **Date:** 10/4/2014

APPROVAL SIGNATURES:

VETERINARY PRE-REVIEW: *Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian before the changes are submitted to the IACUC for review.*

Attending Veterinarian Printed Name: [redacted]

Attending Veterinarian Signature: **Date:**

FINAL IACUC APPROVAL: *All revisions must be approved by the IACUC prior to implementation.*

IACUC Chair or Vice Chair Printed Name:

IACUC Chair or Vice Chair Signature: **Date:**

USDA Pain Code B = Breeding or holding colony: no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

EXPERIMENTAL AGENTS:

Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _____
_____

INVESTIGATOR ASSURANCES:

X	*I understand that it is my responsibility as the Principal Investigator to ensure that all personnel who work on this protocol are adequately trained. That training may be provided by me, an experienced member of the investigative staff, the Attending Veterinarian, a qualified Division of Comparative Medicine (CompMed) staff member, or an appropriate outside source. I hereby affirm that each person added to this protocol by way of this amendment request has been adequately trained to perform the procedure(s) indicated above. Any person who has not been adequately trained will be so trained prior to performing the procedure(s).*
X	*No animal involved in this project will be subjected to discomfort, pain, or distress, unless it is unavoidable in the conduct of scientifically valuable research and approval of such has been approved by the IACUC.*
X	*I agree to comply with all federal and institutional policies governing the use of animals used in this project.*

PRINCIPAL INVESTIGATOR:

Printed Name: Gerald J Pepe, Ph.D.

Signature: Gerald J Pepe **Date:** 10/06/2014

APPROVAL SIGNATURES:

VETERINARY PRE-REVIEW: *Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian before the changes are submitted to the IACUC for review.*

Attending Veterinarian Printed Name: [redacted]

Attending Veterinarian Signature: [redacted] **Date:** 10/7/14

FINAL IACUC APPROVAL: *All revisions must be approved by the IACUC prior to implementation.*

IACUC Chair or Vice Chair Printed Name: [redacted]

IACUC Chair or Vice Chair Signature: [redacted] **Date:** Oct. 20, 2014

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

EVMS
Eastern Virginia Medical School

June 10, 2015

Gerald J. Pepe, Ph.D.
Chair, Department of Physiological Sciences
Eastern Virginia Medical School
[redacted]
Norfolk, Virginia 23507

Dear Dr. Pepe:

The Institutional Animal Care and Use Committee reviewed your request to amend the protocol entitled, ***Regulation of Fetal-placental Development in the Primate*** **(IACUC #12-010)**, at its June 4, 2015 meeting. **The following amendments to the protocol were approved:**

1. **permission to place a small single knot in the fascia at the muscle biopsy site using a non-absorbable suture to serve as a point of reference for future experiments as the animal grows, and**
2. **permission to administer the oral analgesic agent, Ketoprofen (50 mg/capsule, PO), following the glucose tolerance test (GTT) with muscle biopsy and for 2 additional days SID (once a day).**

Although the Division of Comparative Medicine (CompMed) will make every effort to provide housing, husbandry, and veterinary support for your research project, IACUC approval of this amendment request does not guarantee that CompMed will be able to accommodate the work immediately. Available space and resources within the CompMed animal facility are determined by the number of ongoing projects and the animal census at any given time. Please consult with the CompMed management team to confirm availability prior to implementing the approved modification(s).

It is the responsibility of the Principal Investigator to notify the Committee, in writing, of any deviation from the IACUC-approved protocol. Please note that a change in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian prior to submitting the amendment request to the IACUC for approval.

It is also the responsibility of the Principal Investigator to provide for regular observation of the health of the research animals and to provide the Division of Comparative Medicine (CompMed) with the name(s) and telephone number(s) of the person(s) who may be contacted and/or take action in the event that the CompMed staff notes an emergency condition with the research animals.

Thank you for your continued cooperation with the Institutional Animal Care and Use Committee.

Sincerely,

[redacted]
Chair
Institutional Animal Care and Use Committee

[redacted]

cc: [redacted]
Attending Veterinarian
Division of Comparative Medicine

[redacted]
Project Manager
Division of Comparative Medicine

[redacted]
Senior Associate Dean for Research
Institutional Official

April 2010
Updated February 2011; March 2011; April 2012; August 2013

REQUEST TO AMEND A PREVIOUSLY APPROVED IACUC PROTOCOL

Institutional Animal Care and Use Committee (IACUC)

APR 29 2015

Eastern Virginia Medical School
Office of Research

IACUC Office
[redacted]

Office: 757-[redacted]
Fax: 757[redacted]

FOR OFFICE USE ONLY
Date Received: 4/29/15 [redacted]
IBC Approval? X Yes / ____ No
Review Method: X FCR / ____ FR / ____ Administrative (*Personnel Changes Only*)
IBC Approval Date: 6/2014
Final Approval Date: 4/[redacted]

General Information and Instructions:

1. All requested amendments must be approved by the IACUC **before** they are implemented.
2. Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian **before** the changes are submitted to the IACUC Office.
3. A request to increase the number of animals assigned to a protocol must be approved by the IACUC **before** the animals are ordered.
4. **Submit the original signed amendment form to the IACUC Office located in [redacted] and e-mail the MSWord version of the form to the IACUC Administrator.**
5. ***The IACUC reserves the right to require submission of an IACUC "Initial Review Form" based upon the Committee's assessment of the amendment(s) requested herein.***

I. ADMINISTRATIVE INFORMATION

Protocol Number: 12-010	**Protocol Initial Approval Date: July 2012** (7/20/12) [redacted]
Protocol Title: Regulation of Fetal-placental Development in the Primate	
Principal Investigator: Gerald J. Pepe	
Approved USDA Pain Code Level(s) – *not required for personnel additions only*: D & B [redacted]	

II. PERSONNEL

List all personnel to be added to or removed from the protocol. ***All persons authorized to work on the protocol must have completed the required CITI/LATA and OHSP training modules and must have completed a health/risk assessment with the Occupational Health Department.***

Name	Added (+) or Removed (-)	E-mail Address *(additions only)*	CITI/LATA Training Certification Number *(additions only)*	CITI/LATA Training Species Module(s) Completed *(additions only)*	OHSP Training Certification Number *(additions only)*	Occupational Health/ Risk Assessment Date *(additions only)*
1.						
2.						
3.						
Procedure(s) to be performed by personnel addition #1:						
Procedure(s) to be performed by personnel addition #2:						
Procedure(s) to be performed by personnel addition #3:						

III. PROPOSED CHANGES TO THE ORIGINALLY APPROVED PROTOCOL

☐ **A. CHANGE OF SPONSOR/FUNDING SOURCE**

List the new/approved funding source, the project/grant number, the project/grant title (if different from the IACUC protocol title), and the award period.

☐ **B. CHANGE IN PROJECT SITE(S)**

List all new locations (i.e., building and room number(s)) and indicate what procedure(s) will be performed in each new location.

☐ **C. CHANGE OF SPECIES AND/OR STRAIN**

List the species to be added to or removed from the protocol. Please provide the rationale for any species/strain additions and indicate the USDA Pain Category assignment for each new species/strain. Also, list any anticipated changes in veterinary care and/or husbandry due to the new species/strain.

Species and/or strain	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E

☐ **D. CHANGE IN ANIMAL NUMBERS**

List the number of animals to be added to or removed from the protocol. If animals will be added, please provide the justification for the increase and indicate the number of animals to be assigned to each USDA Pain Category.

Species and/or strain	Year (1, 2 or 3)	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E	TOTAL

Will the animals be ordered through CompMed? _____Yes _____ No ***(Identify the source and provide the rationale/justification below.)***

Will the animals require special housing (e.g., specific bedding requirements, isolator cages, special feed, etc.)
_____ Yes ***(Please specify below.)*** _____ No

USDA Pain Code B *= Breeding or holding colony; no animal manipulation.*
USDA Pain Code C *= Procedures involving no or momentary slight pain or distress for which no pain-relieving drugs are used.*
USDA Pain Code D *= Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.*
USDA Pain Code E *= Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.*

[X] **E. CHANGE IN EXPERIMENTAL PROCEDURES AND/OR INSTRUMENTATION**

Describe, in detail, the proposed change(s). *Please indicate potentially painful/distressing effects on the animals as a result of the proposed change(s). Also, please indicate any expected adverse effects and any changes in monitoring, husbandry, housing, early endpoint, and/or experimental endpoint. Finally, please complete the checklist below.*

1)We request permission to place a small single knot in the fascia using non-absorbable suture at the muscle biopsy site. This is to act as a reference point for future experiments as the animal grows. Aseptic technique will be followed as already approved in the 12-010 protocol.

2) We request permission to use oral OR injectable analgesia following the GTT with muscle biopsy.

Please check all that apply to the proposed change(s). Complete the required IACUC Attachment and submit it along with the amendment form.	
Biohazardous Agents: ____ Yes __x__ No (e.g., recombinant DNA, RNA, all tissue or cell samples, laboratory-induced infection, cultured pathogens)	Complete and submit **Attachment D**
Radioisotopes, *in vivo*: ____ Yes __x__ No	Complete and submit **Attachment D**
Chemical Agents: ____ Yes __x__ No (e.g., known or suspected chemical hazards, mutagens, or teratogens)	Complete and submit **Attachment D**
Stress or Prolonged Restraint: ____ Yes __x__ No	Complete and submit **Attachment C**
Food and/or Water Deprivation: ____ Yes __x__ No	Complete and submit **Attachment C**
Surgical Procedures: ____ Yes __x__ No (i.e., single survival, multiple survival, or non-survival)	(Complete and submit **Attachment E** Already approved
Antibody Production: ____ Yes __x__ No	Complete and submit **Attachment F**
Toxicity Testing (LD50): ____ Yes __x__ No	Complete and submit **Attachment G**
Lasers or Penetrating Electromagnetic Radiation: ____ Yes __x__ No	
Collection of Tissues, Cells, or Organs: ____ Yes __x__ No (Please explain below.)	
Tumor Transplantation or Induction: ____ Yes __x__ No	
Special Diet: ____ Yes __x__ No (Please explain below.)	
Other : N/A (Please specify below.)	

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

[X] **F. CHANGE IN ANESTHESIA, THERAPEUTICS, AND/OR EXPERIMENTAL AGENTS**

	Dose (mg/kg)	Route of Administration	Frequency of Administration	
Sedatives/Tranquilizers				
Anesthetics - General				
Anesthetics - Local				
				Length of Administration
Analgesics				
Ketoprofen	50mg/capsule	PO	Completion of GTT with muscle biopsy +2days SID	3days total (PRN with vet consult)
Antibiotics				
Miscellaneous				

EXPERIMENTAL AGENTS:

Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _____
_____

Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _____
_____

Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _____
_____

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

INVESTIGATOR ASSURANCES:	
X	*I understand that it is my responsibility as the Principal Investigator to ensure that all personnel who work on this protocol are adequately trained. That training may be provided by me, an experienced member of the investigative staff, the Attending Veterinarian, a qualified Division of Comparative Medicine (CompMed) staff member, or an appropriate outside source. I hereby affirm that each person added to this protocol by way of this amendment request has been adequately trained to perform the procedure(s) indicated above. Any person who has not been adequately trained will be so trained prior to performing the procedure(s).*
X	*No animal involved in this project will be subjected to discomfort, pain, or distress, unless it is unavoidable in the conduct of scientifically valuable research and approval of such has been approved by the IACUC.*
X	*I agree to comply with all federal and institutional policies governing the use of animals used in this project.*

PRINCIPAL INVESTIGATOR:	
Printed Name:	
Signature:	**Date:** 4/21/2015

APPROVAL SIGNATURES:	
VETERINARY PRE-REVIEW: *Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian before the changes are submitted to the IACUC for review.* **Attending Veterinarian Printed Name:** [redacted] **Attending Veterinarian Signature:** [redacted]	**Date:** 4/7/15
FINAL IACUC APPROVAL: *All revisions must be approved by the IACUC prior to implementation.* **IACUC Chair or Vice Chair Printed Name:** [redacted] **IACUC Chair or Vice Chair Signature:** [redacted]	June 15, 2015 **Date:**

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

OFFICE OF RESEARCH | WWW.EVMS.EDU
[redacted] ☐ Norfolk, VA 23507 | Phone: 757[redacted] ☐ Fax [redacted]

EVMS
Eastern Virginia Medical School

December 7, 2012

Gerald J. Pepe, Ph.D.
Chair, Department of Physiological Sciences
Eastern Virginia Medical School
[redacted]
Norfolk, Virginia 23507

Dear Dr. Pepe: Jerry

The Institutional Animal Care and Use Committee reviewed your request to amend the protocol entitled, ***Regulation of Fetal-placental Development in the Primate*** **(IACUC #12-010)**, at its December 6, 2012 meeting. **The following amendments to the protocol were approved:**

1. **permission to perform a terminal intravenous glucose tolerance test (IVGTT) and obtain biopsies of skeletal muscle and adipose tissues prior to initial of the IVGTT (experimental time 0) and at experimental time 20 minutes (after the bolus infusion of glucose). It is the understanding of the Committee that the animal will be euthanized at experimental time 90 minutes and that tissues will be harvested as outlined in the approved protocol.**
2. **addition of [redacted] to advise on tissue collection and fixation and to perform experimental analyses of the collected specimens.**

Although the Division of Comparative Medicine (CompMed) will make every effort to provide housing, husbandry, and veterinary support for your research project, IACUC approval of this amendment request does not guarantee that CompMed will be able to accommodate the work immediately. Available space and resources within the CompMed animal facility are determined by the number of ongoing projects and the animal census at any given time. Please consult with the CompMed management team to confirm availability prior to implementing the approved modification(s).

It is the responsibility of the Principal Investigator to notify the Committee, in writing, of any deviation from the IACUC-approved protocol. Please note that a change in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian prior to submitting the amendment request to the IACUC for approval.

It is also the responsibility of the Principal Investigator to provide for regular observation of the health of the research animals and to provide the Division of Comparative Medicine (CompMed) with the name(s) and telephone number(s) of the person(s) who may be contacted and/or take action in the event that the CompMed staff notes an emergency condition with the research animals.

Thank you for your continued cooperation with the Institutional Animal Care and Use Committee.

Sincerely,

[redacted]

[redacted] *Chair*
Institutional Animal Care and Use Committee

[redacted]

cc: [redacted]
Attending Veterinarian
Division of Comparative Medicine

[redacted]
Program Manager
Division of Comparative Medicine

[redacted]
Associate Dean for Research
Institutional Official

PHYSIOLOGICAL SCIENCES | WWW.EVMS.EDU
P.O. Box 1980 Norfolk, VA 23501-1980 | Phone: 757[redacted] Fax: 757[redacted]

EVMS
Eastern Virginia Medical School

NOV 27 2012

November 27, 2012

Gerald J. Pepe, PhD
Professor and Chair

[redacted] Chair
Institutional Animal Care and Use Committee
Eastern Virginia Medical School
Norfolk, VA 23507

Dear [redacted]

I would like to add the following terminal procedure to my approved IACUC protocol, entitled "Regulation of Fetal-Placental Development in the Primate" (#12-010). Briefly, for those animals that have completed study of the role of estrogen in pregnancy and are scheduled to be euthanized, I am requesting to [1] perform a terminal intravenous glucose tolerance test (IVGTT) and [2] obtain biopsies of skeletal muscle and adipose tissue prior to initiation of the IVGTT (experimental time 0) and at experimental time 20 min (i.e. 20 minutes after bolus infusion of glucose). Animals will then be euthanized (experimental time 90 min) and tissues harvested per approved protocol. The baboons to be used for this terminal study have completed the approved experimental protocols (e.g. have had the maximum number of survival surgeries) and cannot be transferred to another protocol and thus are approved to be euthanized.

In addition, I would like to request the addition of [redacted]., Associate Professor, department of Physiological Sciences EVMS to my protocol. [redacted] is an expert on adipose tissue biology/biochemistry and a co-investigator on this aspect of my research program. [redacted] will advise us on tissue collection and fixation and perform experimental analyses on these specimens.

The IACUC's time and effort in reviewing this amendment are most appreciated.

Sincerely,

Gerald J. Pepe, Ph.D.

GJP/[redacted]

April 2010
Updated February 2011; March 2011; April 2012

REQUEST TO AMEND A PREVIOUSLY APPROVED IACUC PROTOCOL

NOV 27 2012

Institutional Animal Care and Use Committee (IACUC)

Eastern Virginia Medical School
Office of Research

IACUC Office
[redacted]

Office: 757[redacted]
Fax: 757-[redacted]

FOR OFFICE USE ONLY [redacted]
Date Received: 11/27/12 **Review Method:** X FCR / ___ FR / ___ Administrative (*Personnel Changes Only*)
IBC Approval? (Yes / ___ No **IBC Approval Date:** 01-014 **Final Approval Date:** 12/6/12 [redacted]

General Information and Instructions:

1. All requested amendments must be approved by the IACUC **before** they are implemented.
2. Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian **before** the changes are submitted to the IACUC Office.
3. A request to increase the number of animals assigned to a protocol must be approved by the IACUC **before** the animals are ordered.
4. **Submit the original signed amendment form to the IACUC Office located in** [redacted], **and e-mail the MSWord version of the form to the IACUC Administrator.**
5. ***The IACUC reserves the right to require submission of an IACUC "Initial Review Form" based upon the Committee's assessment of the amendment(s) requested herein.***

I. ADMINISTRATIVE INFORMATION

Protocol Number: 12-010	**Protocol Initial Approval Date: 7/30/12**
Protocol Title: Regulation of Fetal-Placental Development in the Primate	
Principal Investigator: Gerald J. Pepe	
Approved USDA Pain Code Level(s) – *B and D*	

II. PERSONNEL

List all personnel to be added to or removed from the protocol. All persons authorized to work on the protocol must have completed the required CITI/LATA and OHSP training modules and must have completed a health/risk assessment with the Occupational Health Department.

Name	Added (+) or Removed (-)	E-mail Address *(additions only)*	CITI/LATA Training Certification Number *(additions only)*	CITI/LATA Training Species Module(s) Completed *(additions only)*	OHSP Training Certification Number *(additions only)*	Occupational Health/ Risk Assessment Date *(additions only)*
1. [redacted]	+		[redacted]	Rodent / NHP	[redacted]	11/20/2012
2.						
3.						

Procedure(s) to be performed by personnel addition #1: assist in tissue collection and fixation

Procedure(s) to be performed by personnel addition #2:

Procedure(s) to be performed by personnel addition #3:

III. PROPOSED CHANGES TO THE ORIGINALLY APPROVED PROTOCOL

☐ **A. CHANGE OF SPONSOR/FUNDING SOURCE**

List the new/approved funding source, the project/grant number, the project/grant title (if different from the IACUC protocol title), and the award period.

☐ **B. CHANGE IN PROJECT SITE(S)**

List all new locations (i.e., building and room number(s)) and indicate what procedure(s) will be performed in each new location.

☐ **C. CHANGE OF SPECIES AND/OR STRAIN**

List the species to be added to or removed from the protocol. Please provide the rationale for any species/strain additions and indicate the USDA Pain Category assignment for each new species/strain. Also, list any anticipated changes in veterinary care and/or husbandry due to the new species/strain.

Species and/or strain	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E

☐ **D. CHANGE IN ANIMAL NUMBERS**

List the number of animals to be added to or removed from the protocol. If animals will be added, please provide the justification for the increase and indicate the number of animals to be assigned to each USDA Pain Category.

Species and/or strain	Year (1, 2 or 3)	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E	TOTAL

Will the animals be ordered through CompMed? _____Yes _____ No ***(Identify the source and provide the rationale/justification below.)***

Will the animals require special housing (e.g., specific bedding requirements, isolator cages, special feed, etc.)
_____ Yes ***(Please specify below.)*** _____ No

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

[X] **E. CHANGE IN EXPERIMENTAL PROCEDURES AND/OR INSTRUMENTATION**

Describe, in detail, the proposed change(s). *Please indicate potentially painful/distressing effects on the animals as a result of the proposed change(s). Also, please indicate any expected adverse effects and any changes in monitoring, husbandry, housing, early endpoint, and/or experimental endpoint. Finally, please complete the checklist below.*

I would like to add the following terminal procedure to my approved IACUC protocol. Briefly, for those animals that have completed study of the role of estrogen in pregnancy and are scheduled to be euthanized, I am requesting to [1] perform a terminal intravenous glucose tolerance test (IVGTT) and [2] obtain biopsies of skeletal muscle and adipose tissue prior to initiation of the IVGTT (experimental time 0) and at experimental time 20 min (i.e. 20 minutes after bolus infusion of glucose). Animals will then be euthanized and tissues harvested per approved protocol. The baboons to be used for this terminal study have completed the approved experimental protocols (e.g. have had the maximum number of survival surgeries) and cannot be transferred to another protocol and thus are approved to be euthanized.

Briefly, animals will be prepped and monitored as outlined in the current approved protocol and IVGTT will be performed as currently approved. At experimental time 0, i.e. before infusion of glucose, the abdomen will be cleaned with betadine and alcohol and an incision will be made through the abdomen and a biopsy of visceral adipose tissue (approximately 1.0 gram) collected. The abdomen will be closed (clamped) and aliquots of adipose placed in fixative or frozen in liquid nitrogen. A biopsy of skeletal muscle (vastus lateralis) will then be obtained as outlined in our IACUC approved protocol. Briefly, after washing the skin with betadine and alcohol, a small 1-2 inch incision will be made in the thigh and 6-10mm punch biopsy obtained and the incision clamped. After collection of the muscle biopsy, the IVGTT is initiated and biopsies of adipose and skeletal muscle obtained at experimental time 20 min and incisions clamped. After completion of the IVGTT, i.e. at experimental time 90 min, as outlined in the approved protocol, the animal will be admisterd Fatal Plus IV and once cessation is confirmed, tissues will be harvested as currently approved. Throughout the procedure, the animal is under iv ketamine anesthesia (infusion/dose adjusted as necessary) and vitals/anesthesia monitored throughout the procedure as currently approved.

Finally, I would like to request the addition of [redacted] to my protocol. [redacted] is an expert on adipose tissue biology/biochemistry and a co-investigator on this aspect of our research program. [redacted] will be an advisor in adipose tissue collection and fixation.

Please check all that apply to the proposed change(s). Complete the required IACUC Attachment and submit it (i.e., the original and 17 photocopies) along with the amendment form.	
Biohazardous Agents: ____ Yes _X___ No (e.g., recombinant DNA, RNA, all tissue or cell samples, laboratory-induced infection, cultured pathogens)	Complete and submit **Attachment D**
Radioisotopes, *In vivo*: ____ Yes __X__ No	Complete and submit **Attachment D**
Chemical Agents: ____ Yes __X__ No (e.g., known or suspected chemical hazards, mutagens, or teratogens)	Complete and submit **Attachment D**
Stress or Prolonged Restraint: ____ Yes ___X_ No	Complete and submit **Attachment C**
Food and/or Water Deprivation: ____ Yes _X___ No	Complete and submit **Attachment C**

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

Surgical Procedures: ____ Yes _X___ No (i.e., single survival, multiple survival, or non-survival)	Complete and submit **Attachment E**
Antibody Production: ____ Yes _X___ No	Complete and submit **Attachment F**
Toxicity Testing (LD50): ____ Yes _X___ No	Complete and submit **Attachment G**
Lasers or Penetrating Electromagnetic Radiation: ____ Yes _X___ No	
Collection of Tissues, Cells, or Organs: _X___ Yes ____ No (Please explain below.) I request to take 2 muscle and 2 adipose tissue biopsies before and during a terminal IVGTT test.	
Tumor Transplantation or Induction: ____ Yes __X__ No	
Special Diet: ____ Yes __X__ No (Please explain below.)	
Other : (Please specify below.)	

☐ **F. CHANGE IN ANESTHESIA, THERAPEUTICS, AND/OR EXPERIMENTAL AGENTS**

	Dose (mg/kg)	Route of Administration	Frequency of Administration	Length of Administration
Sedatives/Tranquilizers				
Anesthetics - General				
Anesthetics - Local				
Analgesics				
Antibiotics				
Miscellaneous				

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

EXPERIMENTAL AGENTS:

Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _____
_____

Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _____
_____

Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _____
_____

INVESTIGATOR ASSURANCES:

[X]	*I understand that it is my responsibility as the Principal Investigator to ensure that all personnel who work on this protocol are adequately trained. That training may be provided by me, an experienced member of the investigative staff, the Attending Veterinarian, a qualified Division of Comparative Medicine (CompMed) staff member, or an appropriate outside source. I hereby affirm that each person added to this protocol by way of this amendment request has been adequately trained to perform the procedure(s) indicated above. Any person who has not been adequately trained will be so trained prior to performing the procedure(s).*
[X]	*No animal involved in this project will be subjected to discomfort, pain, or distress, unless it is unavoidable in the conduct of scientifically valuable research and approval of such has been approved by the IACUC.*
[X]	*I agree to comply with all federal and institutional policies governing the use of animals used in this project.*

PRINCIPAL INVESTIGATOR:

Printed Name: Gerald J. Pepe, Ph.D.

Signature: Gerald J. Pepe **Date:** 11/27/2012

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
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USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian before the changes are submitted to the IACUC for review. **Attending Veterinarian Printed Name:** [redacted] **Attending Veterinarian Signature:** [redacted]	**Date:** 11/27/12
FINAL IACUC APPROVAL: *All revisions must be approved by the IACUC prior to implementation.* **IACUC Chair or Vice Chair Printed Name:** [redacted] **IACUC Chair or Vice Chair Signature:** [redacted]	**Date:** 12/22/12

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

OFFICE OF RESEARCH | WWW.EVMS.EDU
[redacted] Norfolk, VA 23507 | Phone: 757 [redacted] Fax: 757 [redacted]

EVMS
Eastern Virginia Medical School

June 7, 2013

Gerald J. Pepe, Ph.D.
Chair, Department of Physiological Sciences
Eastern Virginia Medical School
[redacted]
Norfolk, Virginia 23507

Dear Dr. Pepe: Jerry

The Institutional Animal Care and Use Committee reviewed your request to amend the protocol entitled, ***Regulation of Fetal-placental Development in the Primate*** **(IACUC #12-010)**, at its June 6, 2013 meeting. **The following amendments to the protocol were approved:**

1. **addition of [redacted] to perform ABR hearing tests in the animals.**
2. **removal of Acetylcholine @ 2 µg/kg and addition of an infusion dose of Acetylcholine @ 4 and 8 µg/kg BW/min/0.3 ml saline to determine its effect on vascular function**
3. **use of chemical-grade N-nitro-L-arginine methyl ester (L-NAME) @ 40 and 67 µg/kg BW/min/0.3 ml saline to examine its concomitant IV infusion effects. It is the understanding of the Committee that vascular function will be examined in the presence of Acetylcholine. The Committee further understands that noninvasive brachial artery flow-mediated dilation and volumetric flow will be assessed by Doppler ultrasonography.**

Although the Division of Comparative Medicine (CompMed) will make every effort to provide housing, husbandry, and veterinary support for your research project, IACUC approval of this amendment request does not guarantee that CompMed will be able to accommodate the work immediately. Available space and resources within the CompMed animal facility are determined by the number of ongoing projects and the animal census at any given time. Please consult with the CompMed management team to confirm availability prior to implementing the approved modification(s).

It is the responsibility of the Principal Investigator to notify the Committee, in writing, of any deviation from the IACUC-approved protocol. Please note that a change in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian prior to submitting the amendment request to the IACUC for approval.

It is also the responsibility of the Principal Investigator to provide for regular observation of the health of the research animals and to provide the Division of Comparative Medicine (CompMed) with the name(s) and telephone number(s) of the person(s) who may be contacted and/or take action in the event that the CompMed staff notes an emergency condition with the research animals.

Thank you for your continued cooperation with the Institutional Animal Care and Use Committee.

Sincerely,

[redacted]

[redacted] *Chair*
Institutional Animal Care and Use Committee

[redacted]

cc: [redacted]
Attending Veterinarian
Division of Comparative Medicine

[redacted]
Program Manager
Division of Comparative Medicine

[redacted]
Associate Dean for Research
Institutional Official

PHYSIOLOGICAL SCIENCES | WWW.EVMS.EDU
P.O. Box 1980 ☐ Norfolk, VA 23501-1980 | Phone: 757 [redacted] ☐ Fax: 757 [redacted]

EVMS
Eastern Virginia Medical School

Gerald J. Pepe, PhD
Professor and Chair

May 20, 2013

[redacted] Chair
Institutional Animal Care and Use Committee
Eastern Virginia Medical School
Norfolk, VA 23507

Dear [redacted]

I would like to have the appended research protocol (briefly outlined below) amended to my approved IACUC protocol entitled "Regulation of Fetal-Placental Development in the Primate" (#12-010). In the existing protocol, we are approved to determine the effects of pharmacologic agents e.g. acetylcholine on vascular function (blood pressure, heart rate, brachial artery diameter and flow) in baboon neonates/adolescents born to mothers untreated or treated in utero with estradiol early in gestation. The goal of these studies is to test the hypothesis that early estradiol treatment programs fetal vascular endothelial function manifest as generalized endothelial dysfunction, i.e. decreased endothelial nitric oxide-dependent vasodilation in adulthood. Briefly, baboon neonates/adolescents are anesthetized with ketamine and sedated with isoflurane and flow mediated parameters measured before and during a 30 min intravenous infusion of acetylcholine at 2 and 4 μg/min/kg body weight/0.3 ml saline. To address concerns raised by experts in the area of vascular biology, I am requesting the following amendments to the protocol: [1] eliminate the 2 μg dose and add infusion of a dose of acetylcholine of 8 μg/kg/BW/0.3 ml/min; [2] examine the effects of concomitant iv infusion of N-nitro-L-arginine methyl ester (L-NAME), an inhibitor of endothelial nitric oxide synthase or nitric oxide (NO) typically increased by acetylcholine; [3] permission to use chemical grade L-NAME.

Accordingly, we are requesting to examine vascular function before (0 time) and during infusion of acetylcholine at 4 and then 8 μg/kg bw/0.3 ml saline over a total of 10 minutes, followed by a 10 minute wash out with saline and then infusion of acetylcholine at 4 and then 8 μg/kg bw/0.3 ml saline in the presence of concomitant intravenous infusion of L-NAME (40 μg/kg bw/min) initiated at the start of the saline wash-out (total dose of 0.8 mg/kg bw). Studies using intravenous infusion of L-NAME at a dose of 67 μg//kg bw/min (total dose of 4 mg/kg bw) have been performed in humans without any effects other than those expected (Jones et al, J Physiol, 560:329, 2004; Fransden et al, J Physiol 531:257, 2001; copies appended). For example, in the study of Jones et al, L-NAME infusion for 60 min increased mean arterial blood pressure from 125 ± 3 mmHg to 135 ± 2 (at 30 min) and 138 ± 3 mm HG (60 min) and decreased heart rate from 56 ± 3 bpm to 44 ± 2 and 41 ± 1 bpm at 30-60 min respectively.

Pharmaceutical grade L-NAME is not available. Chemical grade L-NAME was used in the human experiments described above (Jones et al; 2004; Fransden et al, 2001) without any untoward effects and is available from Sigma Aldrich (product information sheet appended).

The IACUC's time and effort in reviewing this amendment are most appreciated.

Sincerely,

Gerald J. Pepe, Ph.D.

April 2010
Updated February 2011; March 2011; April 2012

RECEIVED MAY 21 2013

REQUEST TO AMEND A PREVIOUSLY APPROVED IACUC PROTOCOL

Institutional Animal Care and Use Committee (IACUC)

Eastern Virginia Medical School
Office of Research
IACUC Office
[redacted]
Office: 757-[redacted]
Fax: 757-[redacted]

FOR OFFICE USE ONLY
Date Received: 5/21/13 [redacted] Review Method: X FCR / ___ FR / ___ Administrative (*Personnel Changes Only*)
IBC Approval? X Yes / ___ No IBC Approval Date: 01-Oct Final Approval Date: 6/6/13

[redacted]

General Information and Instructions:

1. All requested amendments must be approved by the IACUC **before** they are implemented.
2. Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian **before** the changes are submitted to the IACUC Office.
3. A request to increase the number of animals assigned to a protocol must be approved by the IACUC **before** the animals are ordered.
4. **Submit the original signed amendment form to the IACUC Office located in [redacted], and e-mail the MSWord version of the form to the IACUC Administrator.**
5. ***The IACUC reserves the right to require submission of an IACUC "Initial Review Form" based upon the Committee's assessment of the amendment(s) requested herein.***

I. ADMINISTRATIVE INFORMATION

Protocol Number: 12-010	**Protocol Initial Approval Date: July 2012** (7/30/12)
Protocol Title: Regulation of Feta-Placental Development in the Primate	
Principal Investigator: Gerald J Pepe Ph.D.	
Approved USDA Pain Code Level(s) – *not required for personnel additions only*: **B and D**	

II. PERSONNEL

List all personnel to be added to or removed from the protocol. ***All persons authorized to work on the protocol must have completed the required CITI/LATA and OHSP training modules and must have completed a health/risk assessment with the Occupational Health Department.***

Name	Added (+) or Removed (-)	E-mail Address *(additions only)*	CITI/LATA Training Certification Number *(additions only)*	CITI/LATA Training Species Module(s) Completed *(additions only)*	OHSP Training Certification Number *(additions only)*	Occupational Health/ Risk Assessment Date *(additions only)*
1. [redacted]	+	[redacted]	[redacted]	NHP	[redacted]	4/17/2013
2.						
3.						

Procedure(s) to be performed by personnel addition #1:
ABR hearing test in baboons

Procedure(s) to be performed by personnel addition #2:

Procedure(s) to be performed by personnel addition #3:

III. PROPOSED CHANGES TO THE ORIGINALLY APPROVED PROTOCOL

☐ **A. CHANGE OF SPONSOR/FUNDING SOURCE**

List the new/approved funding source, the project/grant number, the project/grant title (if different from the IACUC protocol title), and the award period.

☐ **B. CHANGE IN PROJECT SITE(S)**

List all new locations (i.e., building and room number(s)) and indicate what procedure(s) will be performed in each new location.

☐ **C. CHANGE OF SPECIES AND/OR STRAIN**

List the species to be added to or removed from the protocol. Please provide the rationale for any species/strain additions and indicate the USDA Pain Category assignment for each new species/strain. Also, list any anticipated changes in veterinary care and/or husbandry due to the new species/strain.

Species and/or strain	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E

☐ **D. CHANGE IN ANIMAL NUMBERS**

List the number of animals to be added to or removed from the protocol. If animals will be added, please provide the justification for the increase and indicate the number of animals to be assigned to each USDA Pain Category.

Species and/or strain	Year (1, 2 or 3)	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E	TOTAL

Will the animals be ordered through CompMed? _____Yes _____ No ***(Identify the source and provide the rationale/justification below.)***

Will the animals require special housing (e.g., specific bedding requirements, isolator cages, special feed, etc.)
_____ Yes ***(Please specify below.)*** _____ No

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***USDA Pain Code D** = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.*
***USDA Pain Code E** = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.*

[X] **E. CHANGE IN EXPERIMENTAL PROCEDURES AND/OR INSTRUMENTATION**

Describe, in detail, the proposed change(s). *Please indicate potentially painful/distressing effects on the animals as a result of the proposed change(s). Also, please indicate any expected adverse effects and any changes in monitoring, husbandry, housing, early endpoint, and/or experimental endpoint. Finally, please complete the checklist below.*

As outlined in approved protocol 12-010, animals are sedated with ketamine, maintained on isoflurane/O2 for the duration of the experiment. BP, HR and body temp are monitored by comp med staff.

During the infusion, baboons will be placed in a supine position upon a Bear Hugger to maintain body temperature. A sterile 21-gauge catheter inserted into an antecubital or saphenous vein and then an infusion of acetylcholine (4 and 8 µg/kg bw/min/0.3 ml saline) for 10 min per dose to permit the flow measurements described below. Another 21-gauge catheter will be inserted into the other antecubital or saphenous vein

Noninvasive brachial artery flow-mediated dilation and volumetric flow will also be assessed by Doppler ultrasonography as originally approved. Followed by a 10 minute wash out with saline and then infusion of acetylcholine at 4 and then 8 µg/kg bw/0.3 ml saline in the presence of concomitant intravenous infusion of L-NAME (40 µg/kg bw/min) initiated at the start of the saline wash-out (total dose of 0.8 mg/kg bw). Noninvasive brachial artery flow-mediated dilation and volumetric flow will also be assessed by Doppler ultrasonography will then be repeated. Upon final ultrasound reading animal will be off anesthesia and monitored until able to respond to stimuli and then returned to housing cage and monitored until upright.

Please check all that apply to the proposed change(s). Complete the required IACUC Attachment and submit it (i.e., the original and 17 photocopies) along with the amendment form.	
Biohazardous Agents: ____ Yes ____ No (e.g., recombinant DNA, RNA, all tissue or cell samples, laboratory-induced infection, cultured pathogens)	Complete and submit **Attachment D**
Radioisotopes, *in vivo*: ____ Yes ____ No	Complete and submit **Attachment D**
Chemical Agents: ____ Yes ____ No (e.g., known or suspected chemical hazards, mutagens, or teratogens)	Complete and submit **Attachment D**
Stress or Prolonged Restraint: ____ Yes ____ No	Complete and submit **Attachment C**
Food and/or Water Deprivation: ____ Yes ____ No	Complete and submit **Attachment C**
Surgical Procedures: ____ Yes ____ No (i.e., single survival, multiple survival, or non-survival)	Complete and submit **Attachment E**
Antibody Production: ____ Yes ____ No	Complete and submit **Attachment F**
Toxicity Testing (LD50): ____ Yes ____ No	Complete and submit **Attachment G**

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***USDA Pain Code C** = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.*
***USDA Pain Code D** = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.*
***USDA Pain Code E** = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.*

Lasers or Penetrating Electromagnetic Radiation: ____ Yes ____ No	
Collection of Tissues, Cells, or Organs: ____ Yes ____ No (Please explain below.)	
Tumor Transplantation or Induction: ____ Yes ____ No	
Special Diet: ____ Yes ____ No (Please explain below.)	
Other : (Please specify below.)	

X **F. CHANGE IN ANESTHESIA, THERAPEUTICS, AND/OR EXPERIMENTAL AGENTS**

	Dose (mg/kg)	Route of Administration	Frequency of Administration	
Sedatives/Tranquilizers				
Anesthetics - General				
Anesthetics - Local				
				Length of Administration
Analgesics				
Antibiotics				
Miscellaneous				

EXPERIMENTAL AGENTS:

Agent: _____ Acetylcholine _____ **Agent Vehicle:** ___0.9% saline _____
Route/Site: _____IV_____ **Volume per administration:** 4 µg/kg BW/min/0.3 ml saline and then 8 µg/kg BW/min/0.3 ml saline (each for 10min, i.e. in 'step-up' fashion)_____
Frequency of administration: 4 times over the life span of the animal. Not to exceed once a month._____
Expected side effects and/or changes in animal behavior: _ Expected side effects and/or changes in the

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USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

animal's behavior: At this low dose, we do not anticipate changes in behavior or physiological function. HR, BP and body temp are measured throughout the experiment. If there is a consistent decrease or unlikely increase in HR or BP the infusion will be stopped and the veterinarian contacted for treatment options_____

Agent: N-nitro-L-arginine methyl ester (L-NAME) **Agent Vehicle:** 0.9%Saline_____
Route/Site: _____IV___ **Volume per administration:** _40 µg/kg BW/min/0.3 ml saline and then 67 µg/kg BW/min/0.3 ml saline (each for 10min, i.e. in 'step-up' fashion)_____
Frequency of administration: ___4 times over the life span of the animal. Not to exceed once a month.____
Expected side effects and/or changes in animal behavior: __Expected side effects and/or changes in the animal's behavior: At this low dose, we do not anticipate changes in behavior or physiological function. HR, BP and body temp are measured throughout the experiment. If there is a consistent decrease or unlikely increase in HR or BP the infusion will be stopped and the veterinarian contacted for treatment options_____

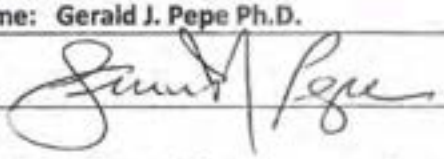
Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _____

INVESTIGATOR ASSURANCES:

X	*I understand that it is my responsibility as the Principal Investigator to ensure that all personnel who work on this protocol are adequately trained. That training may be provided by me, an experienced member of the investigative staff, the Attending Veterinarian, a qualified Division of Comparative Medicine (CompMed) staff member, or an appropriate outside source. I hereby affirm that each person added to this protocol by way of this amendment request has been adequately trained to perform the procedure(s) indicated above. Any person who has not been adequately trained will be so trained prior to performing the procedure(s).*
X	*No animal involved in this project will be subjected to discomfort, pain, or distress, unless it is unavoidable in the conduct of scientifically valuable research and approval of such has been approved by the IACUC.*
X	*I agree to comply with all federal and institutional policies governing the use of animals used in this project.*

PRINCIPAL INVESTIGATOR:

Printed Name: Gerald J. Pepe Ph.D.

Signature:  **Date: May 21, 2013**

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APPROVAL SIGNATURES:	
PRE-VETERINARY REVIEW: *Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian before the changes are submitted to the IACUC for review.* **Attending Veterinarian Printed Name:** [redacted] **Attending Veterinarian Signature:** [redacted]	**Date:** 5/21/2013
FINAL IACUC APPROVAL: *All revisions must be approved by the IACUC prior to implementation.* **IACUC Chair or Vice Chair Printed Name:** [redacted] **IACUC Chair or Vice Chair Signature:** [redacted]	**Date:** 6/10/13

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
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USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

EVMS
Eastern Virginia Medical School

October 3, 2014

Gerald J. Pepe, Ph.D.
Chair, Department of Physiological Sciences
Eastern Virginia Medical School
[redacted]
Norfolk, Virginia 23507

Dear Dr. Pepe:

The Institutional Animal Care and Use Committee reviewed your request to amend the protocol entitled, ***Regulation of Fetal-placental Development in the Primate*** **(IACUC #12-010)**, at its October 2, 2014 meeting. **The following amendment to the protocol was approved: permission to determine the effects and potential benefits of Ketamine (0.5-0.7 mg/kg BW) on obsessive compulsive behaviors (OCB). It is the understanding of the Committee that, initially, the agent will be administered every other day for 2 weeks via an intramuscular injection and animal behaviors will be monitored before, during, and after administration. Subsequently, the agent will be administered via an intramuscular injection at time 0, 48 hours (2 days), and 96 hours (4 days) and behaviors will be recorded for a total of 3 weeks. The Committee further understands that videos will be used to analyze and quantify animal behaviors.**

Although the Division of Comparative Medicine (CompMed) will make every effort to provide housing, husbandry, and veterinary support for your research project, IACUC approval of this amendment request does not guarantee that CompMed will be able to accommodate the work immediately. Available space and resources within the CompMed animal facility are determined by the number of ongoing projects and the animal census at any given time. Please consult with the CompMed management team to confirm availability prior to implementing the approved modification(s).

It is the responsibility of the Principal Investigator to notify the Committee, in writing, of any deviation from the IACUC-approved protocol. Please note that a change in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian prior to submitting the amendment request to the IACUC for approval.

It is also the responsibility of the Principal Investigator to provide for regular observation of the health of the research animals and to provide the Division of Comparative Medicine (CompMed) with the name(s) and telephone number(s) of the person(s) who may be contacted and/or take action in the event that the CompMed staff notes an emergency condition with the research animals.

Thank you for your continued cooperation with the Institutional Animal Care and Use Committee.

Office of Research
[redacted]
NORFOLK, VA 23507
[redacted]
www.evms.edu

Sincerely,

[redacted]
Chair
Institutional Animal Care and Use Committee

[redacted]

[redacted], D.V.M, M.S.
Attending Veterinarian
Division of Comparative Medicine

[redacted]
Project Manager
Division of Comparative Medicine

[redacted]
Senior Associate Dean for Research
Institutional Official

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EVMS
Eastern Virginia Medical School

Gerald J. Pepe, PhD
Professor and Chair

September 19, 2014

[redacted] Chair
Institutional Animal Care and Use Committee
Eastern Virginia Medical School
Norfolk, VA 23507

Dear [redacted]

I would like to have the appended research protocol (briefly outlined below) amended to my approved IACUC protocol entitled "Regulation of Fetal-Placental Development in the Primate" (#12-010). Briefly, we are/have been approved to study the effect of ketamine on alleviating obsessive compulsive behavior (OCB) in baboons. OCB is a potentially major issue in many animal facilities and developing a protocol to alleviate such would be a major accomplishment and most helpful to these nonhuman primates. To date our studies have shown that OCB was markedly reduced within 24h-48h after acute administration over 60 minutes of ketamine (0.5mg/kg BW) intravenously. OCB was however, reinitiated after 5 days. Accordingly, we then examined the effects of chronic exposure to ketamine administered subcutaneously via osmotic mini-pumps (0.5mg/kg BW/hour) for seven days. Unfortunately, OCB was not alleviated using this experimental paradigm. As ketamine was effective when administered acutely but effects were gone after 5 days, we are requesting permission to determine the effects of ketamine (0.5-0.7 mg/kg BW) administered every other day via intramuscular injection (approximately 1 ml saline vehicle) over a period of two weeks and behavior monitored before, during and after administration of ketamine. Intramuscular injection would result in slow release of ketamine over a period of time (30-60 minutes) comparable to an iv infusion and be provided chronically (i.e. days 0, 2 and 4).

The IACUC's time and effort in reviewing this amendment is most appreciated.

Sincerely,

Gerald J. Pepe, Ph.D.
Professor and Chair

GJP[redacted]

April 2010
Updated February 2011; March 2011; April 2012; August 2013

REQUEST TO AMEND A PREVIOUSLY APPROVED IACUC PROTOCOL

Institutional Animal Care and Use Committee (IACUC)

SEP 19 2014

Eastern Virginia Medical School
Office of Research
IACUC Office
[redacted]
Office: 757-[redacted]
Fax: 757-[redacted]

FOR OFFICE USE ONLY [redacted]
Date Received: 9/19/14 **Review Method:** X FCR / ____ FR / ____ Administrative (*Personnel Changes Only*)
IBC Approval? X Yes / ____ No **IBC Approval Date:** 01-09 **Final Approval Date:** 10/2/14 [redacted]

General Information and Instructions:

1. All requested amendments must be approved by the IACUC **before** they are implemented.
2. Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian **before** the changes are submitted to the IACUC Office.
3. A request to increase the number of animals assigned to a protocol must be approved by the IACUC **before** the animals are ordered.
4. **Submit the original signed amendment form to the IACUC Office located in [redacted], and e-mail the MSWord version of the form to the IACUC Administrator.**
5. ***The IACUC reserves the right to require submission of an IACUC "Initial Review Form" based upon the Committee's assessment of the amendment(s) requested herein.***

I. ADMINISTRATIVE INFORMATION

Protocol Number: 12-010	**Protocol Initial Approval Date: July 30, 2012**
Protocol Title: Regulation of Fetal-placental Development in the Primate	
Principal Investigator: Gerald J. Pepe, Ph.D.	
Approved USDA Pain Code Level(s) – *not required for personnel additions only*: **B and D**	

II. PERSONNEL

List all personnel to be added to or removed from the protocol. All persons authorized to work on the protocol must have completed the required CITI/LATA and OHSP training modules and must have completed a health/risk assessment with the Occupational Health Department.

Name	Added (+) or Removed (-)	E-mail Address *(additions only)*	CITI/LATA Training Certification Number *(additions only)*	CITI/LATA Training Species Module(s) Completed *(additions only)*	OHSP Training Certification Number *(additions only)*	Occupational Health/ Risk Assessment Date *(additions only)*
1.						
2.						
3.						
Procedure(s) to be performed by personnel addition #1:						
Procedure(s) to be performed by personnel addition #2:						
Procedure(s) to be performed by personnel addition #3:						

III. PROPOSED CHANGES TO THE ORIGINALLY APPROVED PROTOCOL

☐ **A. CHANGE OF SPONSOR/FUNDING SOURCE**

List the new/approved funding source, the project/grant number, the project/grant title (if different from the IACUC protocol title), and the award period.

☐ **B. CHANGE IN PROJECT SITE(S)**

List all new locations (i.e., building and room number(s)) and indicate what procedure(s) will be performed in each new location.

☐ **C. CHANGE OF SPECIES AND/OR STRAIN**

List the species to be added to or removed from the protocol. Please provide the rationale for any species/strain additions and indicate the USDA Pain Category assignment for each new species/strain. Also, list any anticipated changes in veterinary care and/or husbandry due to the new species/strain.

Species and/or strain	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E

☐ **D. CHANGE IN ANIMAL NUMBERS**

List the number of animals to be added to or removed from the protocol. If animals will be added, please provide the justification for the increase and indicate the number of animals to be assigned to each USDA Pain Category.

Species and/or strain	Year (1, 2 or 3)	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E	TOTAL

Will the animals be ordered through CompMed? _____Yes _____ No ***(Identify the source and provide the rationale/justification below.)***

Will the animals require special housing (e.g., specific bedding requirements, isolator cages, special feed, etc.)
_____ Yes ***(Please specify below.)*** _____ No

***USDA Pain Code B** = Breeding or holding colony; no animal manipulation.*
***USDA Pain Code C** = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.*
***USDA Pain Code D** = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.*
***USDA Pain Code E** = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.*

X **E. CHANGE IN EXPERIMENTAL PROCEDURES AND/OR INSTRUMENTATION**

Describe, in detail, the proposed change(s). *Please indicate potentially painful/distressing effects on the animals as a result of the proposed change(s). Also, please indicate any expected adverse effects and any changes in monitoring, husbandry, housing, early endpoint, and/or experimental endpoint. Finally, please complete the checklist below.*

To date, we have performed an acute and a chronic study to ascertain the effects and potential benefits of ketamine to treat Obsessive Compulsive Behaviors (OCB) in nonhuman primates (NHP). The results of both experimental paradigms have been presented at AAALAS and APV national meetings. In the acute study, ketamine was injected to 3 NHPs iv over a period of one hour at a dose of 0.5 mg/kg BW. Results from this study showed after a 60 minute infusion, all 3 NHPs exhibited a marked decrease in pacing and other OCB. As anticipated, all animals subsequently exhibited OCB comparable to that before infusion of ketamine within approximately 5 days. Thus, in the second study, osmotic mini pumps were implanted SQ to deliver ketamine constantly for 7 days at a dosage of 0.5 mg/kg BW per hour. However, unlike the acute study, constant infusion of the same amount of ketamine had no significant effect on OCB.

Accordingly we are requesting approval to perform an additional study in which Ketamine is administered at a dose of at 0.5-0.7 mg/kg BW administered IM qod for two (2) weeks.

Briefly, baseline behaviors are recorded for one week at 20 minute intervals at the same time each day to reduce variability. Ketamine (0.5-0.7 mg/kg BW; approximately 1 ml saline) will then be given IM and behaviors recorded at 20 minute intervals at the same time each day for the treatment weeks. Since our original acute study indicated that the effect of a single injection of ketamine provided benefits that lasted 48 hours, we will then perform a subsequent experiment in which an IM injection is administered at time 0 and then 48 hours (2 days) and 96 hours (4days) later and behaviors recorded for two weeks (during injection week and ~1 week post injection).

In all experiments, animals will be restrained in home cage by squeeze mechanism. Animals do not need to be NPO'd for this procedure and stress is minimal.

Videos will be analyzed and quantify for negative behaviors such as pacing, over-grooming and aggressive behavior (self or toward others). Base measurements will serve as control.

Our goal is to show that a less invasive treatment can minimize OCB in NHP. Similar to our first study but with more sustainable results and less invasive procedures.

Please check all that apply to the proposed change(s). Complete the required IACUC Attachment and submit it along with the amendment form.	
Biohazardous Agents: ____ Yes __x__ No (e.g., recombinant DNA, RNA, all tissue or cell samples, laboratory-induced infection, cultured pathogens)	Complete and submit **Attachment D**
Radioisotopes, *in vivo*: ____ Yes __x__ No	Complete and submit **Attachment D**
Chemical Agents: ____ Yes __x__ No (e.g., known or suspected chemical hazards, mutagens, or teratogens)	Complete and submit **Attachment D**
Stress or Prolonged Restraint: ____ Yes __x__ No	Complete and submit **Attachment C**
Food and/or Water Deprivation: ____ Yes __x__ No	Complete and submit **Attachment C**
Surgical Procedures: ____ Yes __x__ No (i.e., single survival, multiple survival, or non-survival)	Complete and submit **Attachment E**

***USDA Pain Code B** = Breeding or holding colony; no animal manipulation.*
***USDA Pain Code C** = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.*
***USDA Pain Code D** = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.*
***USDA Pain Code E** = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.*

Antibody Production: ____ Yes __x__ No	Complete and submit **Attachment F**
Toxicity Testing (LD50): ____ Yes __x__ No	Complete and submit **Attachment G**
Lasers or Penetrating Electromagnetic Radiation: ____ Yes __x__ No	
Collection of Tissues, Cells, or Organs: ____ Yes __x__ No (Please explain below.)	
Tumor Transplantation or Induction: ____ Yes __x__ No	
Special Diet: ____ Yes __x__ No (Please explain below.)	
Other : N/A (Please specify below.)	

[X] **F. CHANGE IN ANESTHESIA, THERAPEUTICS, AND/OR EXPERIMENTAL AGENTS**

	Dose (mg/kg)	Route of Administration	Frequency of Administration	
Sedatives/Tranquilizers				
Anesthetics - General				
Anesthetics - Local				
				Length of Administration
Analgesics				
Antibiotics				
Miscellaneous				

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

EXPERIMENTAL AGENTS:

Agent: Ketamine hydrochloride **Agent Vehicle:** +/- 0.9 NaCl for dilution
Route/Site: IM **Volume per administration:** 0.5-0.7 mg/kg in 1ml total volume
Frequency of administration: QOD for 2 weeks followed by 0, 48 and 96 hours for 3 weeks total
Expected side effects and/or changes in animal behavior: Although not observed so far, baboons may show mild sedation and/or excitement

Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _____
_____

Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _____
_____

INVESTIGATOR ASSURANCES:

☐	*I understand that it is my responsibility as the Principal Investigator to ensure that all personnel who work on this protocol are adequately trained. That training may be provided by me, an experienced member of the investigative staff, the Attending Veterinarian, a qualified Division of Comparative Medicine (CompMed) staff member, or an appropriate outside source. I hereby affirm that each person added to this protocol by way of this amendment request has been adequately trained to perform the procedure(s) indicated above. Any person who has not been adequately trained will be so trained prior to performing the procedure(s).*
☐	*No animal involved in this project will be subjected to discomfort, pain, or distress, unless it is unavoidable in the conduct of scientifically valuable research and approval of such has been approved by the IACUC.*
☐	*I agree to comply with all federal and institutional policies governing the use of animals used in this project.*

PRINCIPAL INVESTIGATOR:

Printed Name:

Signature:	**Date:**

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.
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INVESTIGATOR ASSURANCES:	
X	*I understand that it is my responsibility as the Principal Investigator to ensure that all personnel who work on this protocol are adequately trained. That training may be provided by me, an experienced member of the investigative staff, the Attending Veterinarian, a qualified Division of Comparative Medicine (CompMed) staff member, or an appropriate outside source. I hereby affirm that each person added to this protocol by way of this amendment request has been adequately trained to perform the procedure(s) indicated above. Any person who has not been adequately trained will be so trained prior to performing the procedure(s).*
X	*No animal involved in this project will be subjected to discomfort, pain, or distress, unless it is unavoidable in the conduct of scientifically valuable research and approval of such has been approved by the IACUC.*
X	*I agree to comply with all federal and institutional policies governing the use of animals used in this project.*

PRINCIPAL INVESTIGATOR:	
Printed Name: Gerald J Pepe PhD	
Signature: Gerald J Pepe	**Date:** 9/19/2014

APPROVAL SIGNATURES:	
PRE-VETERINARY REVIEW: *Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian before the changes are submitted to the IACUC for review.* **Attending Veterinarian Printed Name:** [redacted] **Attending Veterinarian Signature:**	See attached e-mail dated 9/24/14 **Date:**
FINAL IACUC APPROVAL: *All revisions must be approved by the IACUC prior to implementation.* **IACUC Chair or Vice Chair Printed Name:** [redacted] **IACUC Chair or Vice Chair Signature:**	**Date:** Oct 7, 2014

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OFFICE OF RESEARCH | WWW.EVMS.EDU
[redacted] Norfolk, VA 23507 | Phone: 757[redacted] | Fax: 75[redacted]

EVMS
Eastern Virginia Medical School

March 1, 2013

Gerald J. Pepe, Ph.D.
Chair, Department of Physiological Sciences
Eastern Virginia Medical School
[redacted]
Norfolk, Virginia 23507

Dear Dr. Pepe: Jerry

The Institutional Animal Care and Use Committee (IACUC) has reviewed the requested information regarding your request to amend the protocol entitled, ***Regulation of Fetal-placental Development in the Primate*** **(IACUC #12-010).** Your original request was reviewed by the IACUC at its February 7, 2013 meeting. **The amendment (*i.e., administration of medical-grade Ketamine HCL for 7 days to determine whether it will alter self-injurious behavior (SIB) for more than 48 hours*) has been approved via the designated member review (DMR) process.**

Although the Division of Comparative Medicine (CompMed) will make every effort to provide housing, husbandry, and veterinary support for your research project, IACUC approval of this amendment request does not guarantee that CompMed will be able to accommodate the work immediately. Available space and resources within the CompMed animal facility are determined by the number of ongoing projects and the animal census at any given time. Please consult with the CompMed management team to confirm availability prior to implementing the approved modification(s).

It is the responsibility of the Principal Investigator to notify the Committee, in writing, of any deviation from the IACUC-approved protocol. Please note that a change in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian prior to submitting the amendment request to the IACUC for approval.

It is also the responsibility of the Principal Investigator to provide for regular observation of the health of research animals and to provide the Division of Comparative Medicine (CompMed) with the name(s) and telephone number(s) of the person(s) who may be contacted and/or take action in the event that the CompMed staff notes an emergency condition with the research animals.

Thank you for your continued cooperation with the Institutional Animal Care and Use Committee.

Sincerely,

[redacted]

[redacted] *Chair*
Institutional Animal Care and Use Committee

[redacted]

cc: [redacted]
Attending Veterinarian
Division of Comparative Medicine

[redacted]
Program Manager
Division of Comparative Medicine

[redacted]
Associate Dean for Research
Institutional Official

REQUEST TO AMEND A PREVIOUSLY APPROVED IACUC PROTOCOL

Institutional Animal Care and Use Committee (IACUC)

FEB 1 2013

Eastern Virginia Medical School
Office of Research
IACUC Office
[redacted]
Office: 757[redacted]
Fax: 757[redacted]

FOR OFFICE USE ONLY
Date Received: 1/29/13 (via e-mail) **Review Method:** X FCR / ___ FR / ___ Administrative (*Personnel Changes Only*)
IBC Approval? X Yes / ___ No **IBC Approval Date:** 01-017 **Final Approval Date:** 3/1/13 [redacted]

General Information and Instructions:

1. All requested amendments must be approved by the IACUC **before** they are implemented.
2. Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian **before** the changes are submitted to the IACUC Office.
3. A request to increase the number of animals assigned to a protocol must be approved by the IACUC **before** the animals are ordered.
4. **Submit the original signed amendment form to the IACUC Office located in [redacted], and e-mail the MSWord version of the form to the IACUC Administrator.**
5. ***The IACUC reserves the right to require submission of an IACUC "Initial Review Form" based upon the Committee's assessment of the amendment(s) requested herein.***

I. ADMINISTRATIVE INFORMATION

Protocol Number: 12-010	**Protocol Initial Approval Date: July 30, 2012**
Protocol Title: Regulation of Fetal placental Development in the Primate	
Principal Investigator: Dr. Gerald Pepe	
Approved USDA Pain Code Level(s) – *not required for personnel additions only*: C ✓ Band D [redacted]	

II. PERSONNEL

List all personnel to be added to or removed from the protocol. ***All persons authorized to work on the protocol must have completed the required CITI/LATA and OHSP training modules and must have completed a health/risk assessment with the Occupational Health Department.***

Name	Added (+) or Removed (-)	E-mail Address *(additions only)*	CITI/LATA Training Certification Number *(additions only)*	CITI/LATA Training Species Module(s) Completed *(additions only)*	OHSP Training Certification Number *(additions only)*	Occupational Health/ Risk Assessment Date *(additions only)*
1.						
2.						
3.						
Procedure(s) to be performed by personnel addition #1:						
Procedure(s) to be performed by personnel addition #2:						
Procedure(s) to be performed by personnel addition #3:						

III. PROPOSED CHANGES TO THE ORIGINALLY APPROVED PROTOCOL

☐ **A. CHANGE OF SPONSOR/FUNDING SOURCE**

List the new/approved funding source, the project/grant number, the project/grant title (if different from the IACUC protocol title), and the award period.

☐ **B. CHANGE IN PROJECT SITE(S)**

List all new locations (i.e., building and room number(s)) and indicate what procedure(s) will be performed in each new location.

☐ **C. CHANGE OF SPECIES AND/OR STRAIN**

List the species to be added to or removed from the protocol. Please provide the rationale for any species/strain additions and indicate the USDA Pain Category assignment for each new species/strain. Also, list any anticipated changes in veterinary care and/or husbandry due to the new species/strain.

Species and/or strain	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E

☐ **D. CHANGE IN ANIMAL NUMBERS**

List the number of animals to be added to or removed from the protocol. If animals will be added, please provide the justification for the increase and indicate the number of animals to be assigned to each USDA Pain Category.

Species and/or strain	Year (1, 2 or 3)	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E	TOTAL

Will the animals be ordered through CompMed? _____Yes _____ No ***(Identify the source and provide the rationale/justification below.)***

Will the animals require special housing (e.g., specific bedding requirements, isolator cages, special feed, etc.)
_____ Yes ***(Please specify below.)*** _____ No

USDA Pain Code B = *Breeding or holding colony; no animal manipulation.*
USDA Pain Code C = *Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.*
USDA Pain Code D = *Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.*
USDA Pain Code E = *Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.*

[X] **E. CHANGE IN EXPERIMENTAL PROCEDURES AND/OR INSTRUMENTATION**

Describe, in detail, the proposed change(s). *Please indicate potentially painful/distressing effects on the animals as a result of the proposed change(s). Also, please indicate any expected adverse effects and any changes in monitoring, husbandry, housing, early endpoint, and/or experimental endpoint. Finally, please complete the checklist below.*

Our pilot project to study the effect of low dose ketamine on self-injurious behavior (SIB) in nonhuman primate was approved by the EVMS IACUC April 9, 2012. In this study we quantified SIB in baboons before and over a period of one week following acute infusion of ketamine. Results from this study were quite interesting and showed that after a 60 minute infusion of ketamine (0.5mg/kg body weight), the non human primates exhibited a marked decrease in pacing and other undesired behavior over the subsequent 24-48 hours. However, this improvement was not further sustained. Thus, over the subsequent 5 day period, all animals exhibited behavior comparable to that before ketamine.

Because ketamine is cleared rapidly, it is a very safe drug for use in nonhuman primates. Indeed, our laboratory has previously performed studies to examine hormone metabolism *in vivo* over a 120 min in baboons sedated with ketamine, im (10 mg/kg), and then via iv infusion at 0.3 mg/min/kg (equivalent to 18 mg/kg/hour) with no untoward effects. However while a very safe drug, because biological half-life is so short, it is not surprising that behavior modification was not sustained beyond 48 hours. Nevertheless, we confirmed that SIB was and therefore can be modified by ketamine. Therefore, we are requesting an amendment to our protocol to determine whether sustained delivery of the same dose of ketamine for 7 days in these same animals will alter SIB for a longer period. Specifically, we propose to deliver ketamine sc at a dose of 0.5 mg/kg/hour via Azlet (Model 2ML1) minipumps implanted sc. We have previously implanted Alzet mini pumps sc to deliver hormones to the mother during pregnancy over a period of two weeks again with no untoward effects. To deliver the same low dose of ketamine (0.5 mg/kg BW) for 1 week, we will need to [1] use chemical grade ketamine to achieve an appropriate dilution and volume and [2] implant 4 minipumps in each animal. Therefore, we are also requesting use of medical grade ketamine hydrochloride available from Sigma (Sigma# K2753). By using the medical grade drug, we will also reduce the number of pumps required by 50%, as the maximum concentration of pharmaceutical grade ketamine available is 100 mg/ml. Finally while our laboratory has successfully used ketamine at higher doses over sustained periods of time, literature search on long term constant subcutaneous infusions also identified protocols using up to 100 times our proposed dosage for up to 4 months at a time with no major complications.

Our protocol is already approved for subcutaneous pump implantation; Attachment E details our current aim.

As in our previous approved protocol, behavioral observation will be based on videotaping the animals at specific time intervals and durations. We propose to record animal in cage twice a week for 30 minutes the week before the pumps are implanted (baseline) and for 2 weeks after. Videos will be analyzed and quantify for negative behaviors such as pacing, over-grooming and aggressive behavior (self or toward others). Base measurements will serve as control.

Please check all that apply to the proposed change(s). Complete the required IACUC Attachment and submit it (i.e., the original and 17 photocopies) along with the amendment form.	
Biohazardous Agents: __X__ Yes ____ No (e.g., recombinant DNA, RNA, all tissue or cell samples, laboratory-induced infection, cultured pathogens)	Complete and submit **Attachment D**
Radioisotopes, *in vivo*: ____ Yes _X___ No	Complete and submit **Attachment D**
Chemical Agents: ____ Yes __X__ No (e.g., known or suspected chemical hazards, mutagens, or teratogens)	Complete and submit **Attachment D**
Stress or Prolonged Restraint: ____ Yes _X___ No	Complete and submit **Attachment C**
Food and/or Water Deprivation: ____ Yes __X__ No	Complete and submit **Attachment C**

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USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

Please check all that apply to the proposed change(s). Complete the required IACUC Attachment and submit it (i.e., the original and 17 photocopies) along with the amendment form.	
Biohazardous Agents: _X_ Yes ____ No (e.g., recombinant DNA, RNA, all tissue or cell samples, laboratory-induced infection, cultured pathogens)	Complete and submit **Attachment D**
Radioisotopes, *in vivo*: ____ Yes _X___ No	Complete and submit **Attachment D**
Chemical Agents: _X_ Yes ____ No (e.g., known or suspected chemical hazards, mutagens, or teratogens)	Complete and submit **Attachment D**
Stress or Prolonged Restraint: ____ Yes _X___ No	Complete and submit **Attachment C**
Food and/or Water Deprivation: ____ Yes _X_ No	Complete and submit **Attachment C**
Surgical Procedures: _X___ Yes ____ No (i.e., single survival, multiple survival, or non-survival)	Complete and submit **Attachment E**
Antibody Production: ____ Yes _X___ No	Complete and submit **Attachment F**
Toxicity Testing (LD50): ____ Yes _X_ No	Complete and submit **Attachment G**
Lasers or Penetrating Electromagnetic Radiation: ____ Yes _X_ No	
Collection of Tissues, Cells, or Organs: ____ Yes _X___ No (Please explain below.)	
Tumor Transplantation or Induction: ____ Yes _X___ No	
Special Diet: ____ Yes _X_ No (Please explain below.)	
Other : (Please specify below.)	

[X] **F. CHANGE IN ANESTHESIA, THERAPEUTICS, AND/OR EXPERIMENTAL AGENTS**

	Dose (mg/kg)	Route of Administration	Frequency of Administration
Sedatives/Tranquilizers			
Anesthetics - General			
Isoflurane	**~4-5**	**Inhalant**	**Once for pump**

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USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

			implantation	
Ketamine	10mg/kg	IM	Once before pump removal.	
Anesthetics - Local				
				Length of Administration
Analgesics				
Flunixin meglumine (Banamine®)	5mg/kg	IM	Once peri-operatively and once 24 hours later.	24 hours post-surgery or as needed afterward.
Antibiotics				
Miscellaneous				

EXPERIMENTAL AGENTS:

Agent: Ketamine hydrochloride Agent Vehicle: Sterile water for infusion
Route/Site: Subcutaneous Volume per administration: 40µl/hr
Frequency of administration: continuous infusion for 1 week.
Expected side effects and/or changes in animal behavior: Monkey may show mild sedation and/or excitement. Very high acute or chronic doses of ketamine have been reported to cause cardiac problems but not at the dose we are proposing.

Agent: _____ Agent Vehicle: _____
Route/Site: _____ Volume per administration: _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _____
_____

Agent: _____ Agent Vehicle: _____
Route/Site: _____ Volume per administration: _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _____
_____

INVESTIGATOR ASSURANCES:

X	*I understand that it is my responsibility as the Principal Investigator to ensure that all personnel who work on this protocol are adequately trained. That training may be provided by me, an*

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USDA Pain Code C = Procedures involving no or momentary slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

	experienced member of the investigative staff, the Attending Veterinarian, a qualified Division of Comparative Medicine (CompMed) staff member, or an appropriate outside source. I hereby affirm that each person added to this protocol by way of this amendment request has been adequately trained to perform the procedure(s) indicated above. Any person who has not been adequately trained will be so trained prior to performing the procedure(s).
X	*No animal involved in this project will be subjected to discomfort, pain, or distress, unless it is unavoidable in the conduct of scientifically valuable research and approval of such has been approved by the IACUC.*
X	*I agree to comply with all federal and institutional policies governing the use of animals used in this project.*

PRINCIPAL INVESTIGATOR:

Printed Name: GERALD J. PEPE, Ph.D.

Signature: [signature] **Date:** 1/29/13

APPROVAL SIGNATURES:

PRE-VETERINARY REVIEW: *Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian before the changes are submitted to the IACUC for review.*

Attending Veterinarian Printed Name: Dr. Mario Rodriguez

Attending Veterinarian Signature: [redacted] **Date:** 1/17/2013

FINAL IACUC APPROVAL: *All revisions must be approved by the IACUC prior to implementation.*

IACUC Chair or Vice Chair Printed Name: [redacted]

IACUC Chair or Vice Chair Signature: [redacted] **Date:** 3/4/13

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Eastern Virginia Medical School
Institutional Animal Care and Use Committee
Attachment D: Use of Hazardous Agents

Project Title: Regulation of Fetal Placental Development in the Primate

This form applies to IACUC protocols using materials such as radioisotopes, infectious agents, carcinogens, biohazards, mutagens, teratogens, abortifacents, acutely toxic chemicals, or other potentially hazardous chemicals. Information provided in this form will enable assessment of risk to individuals handling or caring for animals, as well as risk to other animals. **If you need assistance answering any of the questions, please contact the Environmental Health and Safety Office at (757) [redacted].**

The Division of Comparative Medicine (CompMed) must be notified in writing at least one week prior to the use of hazardous agents in animals. The use of hazardous agents in animals is not permitted until approval has been granted by the CompMed Facility Manager. Please reference the CompMed SOP entitled, *Appropriate Notification, Signage and Protocol for Working with Hazardous Agents in Animal Holding Rooms and Procedure Rooms.*

YOU MUST COMPLETE A SEPARATE FORM FOR EACH HAZARDOUS AGENT USED.

1. Please indicate the type of hazardous agent being used. Check all that apply. For microorganisms, please list the Risk Group or Biosafety Level. For chemicals, please list the CAS # and the LD50 from the Material Safety Data Sheet (MSDS).

   ____ Radioactive material (Isotope: _____)
   ____ Infectious agent (Risk Group or Biosafety Level: _____)
   __X__ Acutely toxic chemical (CAS #: _1867-66-9_____, LD50: ___10-16 minutes_____)
   ____ Known or suspected human carcinogen (Chemical Name: _____)
   ____ Known or suspected human mutagen/teratogen (Chemical Name: _____)
   ____ Recombinant DNA/RNA
   ____ Tissue sample (Type: _____)
   ____ Other (*Describe below*):

2. Please provide specific information about the agent:

Complete name
(Include strain for microorganisms): Ketamine hydrochloride

Dose and frequency of administration: 0.5mg/kg/hr in a constant infusion

Concentration: 200mg/ml

Route: SQ Duration of exposure: 1 week

How long will the animal be maintained after administration? Long term

Animal species: Baboon Estimated animal weight: 15kg

3. Is the agent excreted or shed by the animal?

_____ YES *(Indicate the type of excreta and estimated quantity per day)*

X NO

4. Are there documented human risks from exposure to the agent? (Risks may be determined from the MSDS or from references found in the Biosafety Manual.)

X YES *(Complete Questions 4a-4e)* _____ NO *(Skip to Question 5)*

4a. Indicate the route(s) of human exposure:

X Inhalation
X Ingestion
_____ Other (describe below):
X Contact
_____ Parenteral

4b. Describe the risks associated with the agent (mutagen, teratogen, etc.):

Inhalation May be harmful if inhaled. Causes respiratory tract irritation.
Skin May be harmful if absorbed through skin. Causes skin irritation.
Eyes Causes eye irritation.
Ingestion Toxic if swallowed.

4c. Indicate the acutely toxic/infectious dose in humans (in mg/kg or number of organisms):

Acute toxicity: LD50 Oral - rat - 447 mg/kg; d

4d. Describe any genetic changes to the organism and their suspected effects:

N/A

4e. Describe the symptoms of exposure:

Hallucinations., Central nervous system depression, To the best of Sigma -Aldrich knowledge: "the chemical, physical, and toxicological properties have not been thoroughly investigated."

4f. Describe the first aid methods to be taken in the case of exposure:

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.Move out of dangerous area.
If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.
In case of skin contact
Wash off with soap and plenty of water. Consult a physician.
In case of eye contact
Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.
If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

4g. Indicate all personal protection required:

X	Lab coat/dedicated clothing	_____	Apron
X	Gloves	X	Face shield
X	Goggles	X	Respirator
_____	Other (describe below):		

5. Are there risks to other animals in the room or in the animal facility?

_____ YES *(Complete Questions 5a-5d)* X NO *(Skip to Question 6)*

5a. Describe the risk to other animals:

N/A

5b. Indicate the route of animal exposure:

N/A

5c. Describe all methods that will be used to contain the risk factor:

Preparation of the solution from powder will be done under a hood in our lab. Pumps will be loaded with prepared solution using all precautionary measurements (PPE) prior to surgery.

5d. Are special animal care requirements necessary?

_____ YES *(Describe below)* X NO

6. Are special waste or carcass disposal requirements necessary?

_____ YES *(Describe below)* __X__ NO

7. Briefly describe the procedure(s) for administration of the agent and how the agent is metabolized and/or excreted. Include how long the agent is expected to remain in/be shed from the animal, the concentration of agent in any specific organs, etc.

7a. Please indicate whether or not the laboratory personnel and CompMed staff have been informed of these hazards and have received training on proper handling techniques.

	Laboratory Personnel		**CompMed Staff**
__X__	**Yes**	__X__	**Yes**
_____	No	_____	No

If not, please contact the Environmental Health and Safety Office to schedule the proper training.

8. Please provide any other relevant information regarding the agent (e.g., unique containment recommendations):

Eastern Virginia Medical School
Institutional Animal Care and Use Committee

Attachment E: Animal Surgery

Project Title: Regulation of Fetal placental Development in the Primate

Protocol Number: 12-010

All animal use proposals involving surgery must provide specific details of pre-, intra-, and post-operative care and a plan for relief of pain and distress.

A. PRE-OPERATIVE PROCEDURE

1. List, by name and title, the person(s) responsible for evaluating the health status of the animals.

   [redacted] – Research Specialist.. [redacted] – Attending Veterinarian

2. Will food be withheld?

   X YES (Below, please explain why food will be withheld and state how long it will be withheld.) ____ NO

   12 hours prior to procedure.

3. List all **pre-operative anesthetic and/or analgesic agents** to be used (i.e., name and dosage for each agent).

   Flunixin meglumine 5mg/kg IM

4. Briefly describe how the animals will be prepared for surgery.

   The animal will be fasted overnight prior to the morning procedure. A 10cm by 10 cm square area between the shoulder blades will be shaved and prepared aseptically with Betadine scrub and alcohol.

B. ANESTHETIC PROCEDURE

5. Will the animals be anesthetized?

_X_ YES (Complete Questions 6-8.) _____ NO (Below, please explain why the animals will not be anesthetized, then skip to Section C.)

6. List, by name and title, the person(s) who will administer the anesthesia.

[redacted] or [redacted] – CompMed veterinary technicians and [redacted] - attending Veterinarian.

7. List the anesthetic agent(s) to be used (i.e., name and dosage) and the route of administration for each agent. List, by name and title, the person(s) who will keep the anesthesia records.

Isoflurane induction and maintenance by mask. [redacted] or [redacted] – CompMed veterinary technicians –will keep records.

8. Explain how anesthetic recovery will be monitored and list, by name and title, the person(s) who will monitor the recovery.

After completion animals will be placed in their cages and monitored until able fully awake by [redacted] – CompMed veterinary technicians, [redacted] - attending Veterinarian and/ or [redacted] – Research Specialist.

C. POST-OPERATIVE PROCEDURE:

Investigators should refer to the IACUC *Post-Operative Care Guidelines* when developing a post-operative care plan.

***Appropriate post-operative records must be maintained in accordance with professionally accepted veterinary procedures regardless of the location of the animal. The attending veterinarian retains the authority to change post-operative care as necessary to ensure the comfort of the animal.* AWA, Section 13 and 9 CFR, April 14, 1997.**

9. List, by name and title, the person(s) who will monitor **daily post-operative care**.

[redacted] – CompMed veterinary technicians, [redacted] - attending Veterinarian and/ or [redacted] – Research Specialist.

10. List, by name and title, the person(s) who will keep the post-operative records and list the location(s) where the records will be maintained.

[redacted] – CompMed veterinary technicians, [redacted] - attending Veterinarian and/ or [redacted] – Research Specialist. Records maintained on CompMed's office on [redacted] floor.

11. Will post-operative analgesics be administered?

_X_ YES _____ NO (Below, please explain why post-operative analgesia will not be used, then skip to Section D.)

12. Provide the following information for each **post-operative analgesic agent** to be administered:

Agent: Flunixin meglumine (Banamine®)

Dose and Route: 5mg/kg IM Frequency: Once at surgery and 24 hours later

Post-Operative Duration of Care: 2 days

Agent: _____

Dose and Route: _____ Frequency: _____

Post-Operative Duration of Care: _____

Agent: _____

Dose and Route: _____ Frequency: _____

Post-Operative Duration of Care: _____

D. MULTIPLE SURVIVAL SURGERY

All major multiple survival surgery procedures must be conducted in accordance with the IACUC policy entitled, *Multiple Major Survival Surgery in Experimental Animals*.

The Animal Welfare Regulations state that an animal may not undergo more than one major survival surgery unless scientifically justified by the principal investigator, appropriate for the animal's veterinary care, or approved by the USDA Administrator. The IACUC must specifically review and approve every additional surgery to be performed on a single animal.

13. Will the animals be subjected to more than one survival surgery?

X YES (Complete Questions 13a-13b.) _____ NO (Skip to Question 14.)

13a. Please briefly outline the surgical procedures, explain how the surgeries are related, and **justify the need for more than one surgery per animal**.

These are very minor surgeries, one is for implanting the osmotic pumps one if for removal of the pumps.

13b. How many surgeries will each animal undergo?

2 minor procedures

14. Have any animals to undergo major survival surgery in this protocol already undergone major survival surgery in any other IACUC protocol(s)?

_____ YES (Complete Questions 14a-14d.) X NO (Skip to Section E.)

14a. Identify all animals that have undergone prior surgical procedures in another protocol.

14b. Identify all previous procedures performed on the animal(s) identified in Question 14a.

14c. List the IACUC protocol number(s) under which the previous procedures were performed.

14d. In accordance with the Animal Welfare Act, the IACUC must specifically review and approve all requests for the use of animals that have undergone previous survival surgery under another IACUC protocol. **Please justify the need to reuse such animals in this surgical protocol.**

15. Surgery Classification for All Vertebrate Animal Species

SURGERY TYPE DEFINITIONS	Type 0 Surgery Surgical procedures performed with appropriate anesthesia that do not require the use of additional analgesia.	Type I Surgery Surgical procedures that result in mild pain and require pre-emptive use of at least one dose of additional analgesia pre- or perioperatively.	Type II Surgery Surgical procedures that result in moderate pain and require pre-emptive use of at least one dose of pre- or perioperative additional analgesia lasting at least 24-48 hours.	Type III Surgery Surgical procedures or invasive manipulations that result in marked to severe pain and require pre-emptive use of at least one dose of pre- or perioperative additional analgesia lasting at least 72 hours.
EXAMPLES OF PROCEDURES IN EACH SURGERY TYPE CLASSIFICATION	Small skin incision without deeper tissue manipulation; Skin biopsy; Placement of small subcutaneous implants; Tracheal injection; Retro-orbital bleed	Skin incision and deeper tissue manipulation, i.e., muscle incision, muscle biopsy, vessel cannulation (not percutaneous), vessel incision, vasectomy, non-extensive oral surgery and/or tissue manipulation, bone marrow aspiration	Laparotomy without organ manipulation or incision; Craniotomy (small burr hole); Placement of vascular access ports; Orchiectomy; Superficial lymph node biopsy; Thyroidectomy; Skin graft involving partial thickness and minimal body surface area	Laparotomy with significant organ manipulation or incision; Thoracotomy; Thymectomy; Orthopedic surgery to create or repair a bone fracture; Dissection into joints; Large craniotomy; Intraocular or corneal surgery/manipulation; Skin graft involving full thickness, multiple grafts, and/or extensive body surface area; Extensive oral or dental Surgery; Model involving extensive burns or tissue trauma; Parabiosis; Procedure involving infarction of organs with significant sensory innervation

Source Material: University of Michigan, University Committee on Use and Care of Animals, Policy on Analgesic Use in Animals Undergoing Surgery.

15a. Classify each surgical procedure to be performed according to the table listed above. **(Example: Surgery Type: 1; Procedure to be performed: Bone marrow aspiration.)**

Surgery Type: 1 Procedure to be performed: Subcutaneous pump implantation

Surgery Type: 1 Procedure to be performed: Subcutaneous pump removal

Surgery Type: _____ Procedure to be performed: _____

E. SURGICAL PROCEDURES:

PLEASE SUBMIT A SEPARATE COPY OF THIS PAGE FOR EACH SURGICAL PROCEDURE TO BE PERFORMED.
PLEASE MAKE ADDITIONAL COPIES OF THIS PAGE AS NEEDED.

16. Is the procedure addressed on this page a major or a minor surgical procedure? **(The Animal Welfare Regulations define a major surgical procedure as one that penetrates and exposes a body cavity and/or a procedure that produces permanent impairment of physical or physiological functions. The *Guide* defines it as a procedure that penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection. A minor surgical procedure does not expose a body cavity and causes little or no physical impairment.)**

_____ Major surgical procedure X___ Minor surgical procedure

17. Is the procedure survival surgery?

X___ YES _____ NO

18. Indicate the number of the surgical procedure described on this page (i.e., the only procedure to be performed, the 1st surgical procedure, the 2nd surgical procedure, etc.) If the same exact surgical procedure will be performed more than once on a single animal, indicate below how many times the procedure will be performed on the animal. In that case, submit only one copy of this page for the specific procedure to be performed.

1. implantation of subcutaneous pump
2. removal of pumps (same method 2 weeks alter)

19. Indicate the location (*provide a specific building and room number if surgery will be conducted outside of the CompMed animal facility*), the time of day, and the day(s) of the week the surgery will be performed. Please take into consideration the time necessary for care following administration of anesthesia and the surgical procedure.

[redacted] Monday to Wednesday early in the morning.

20. Describe the entire surgical procedure.

A midline 2 cm skin incision will be performed between the scapulas (shoulder blades) of the animal.
A pocket of about 3cm by 3cm will be created by blunt dissection to each side of the incision (total pocket size ~ 6x6 cm).
4 pumps (Azlets Model 2 ML1) will be introduced (or removed) and the incision will be close with Vycril 2-0 or 3-0 using an intradermal continuous pattern. Glue may be used on the outside to help the apposition and healing process.

EVMS
Eastern Virginia Medical School

August 11, 2014

Gerald J. Pepe, Ph.D.
Chair, Department of Physiological Sciences
Eastern Virginia Medical School
[redacted]
Norfolk, Virginia 23507

Dear Dr. Pepe:

The Institutional Animal Care and Use Committee reviewed your request to amend the protocol entitled, ***Regulation of Fetal-placental Development in the Primate*** **(IACUC #12-010)**, at its August 7, 2014 meeting. **The following amendments to the protocol were approved:**

1. **permission to collect skeletal muscle samples using a scalpel blade instead of punch biopsy; the analgesic agent, Banamine®, will be administered post-operatively, and**
2. **permission to collect skeletal muscle samples at Time 0 and Time 30 minutes from alternating legs. It is the understanding of the Committee that animals will undergo biopsy twice during the study (i.e., at 3-4 years of age (pre-pubertal) and at 6-10 years of age (post-puberty)).**

Although the Division of Comparative Medicine (CompMed) will make every effort to provide housing, husbandry, and veterinary support for your research project, IACUC approval of this amendment request does not guarantee that CompMed will be able to accommodate the work immediately. Available space and resources within the CompMed animal facility are determined by the number of ongoing projects and the animal census at any given time. Please consult with the CompMed management team to confirm availability prior to implementing the approved modification(s).

It is the responsibility of the Principal Investigator to notify the Committee, in writing, of any deviation from the IACUC-approved protocol. Please note that a change in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian prior to submitting the amendment request to the IACUC for approval.

It is also the responsibility of the Principal Investigator to provide for regular observation of the health of the research animals and to provide the Division of Comparative Medicine (CompMed) with the name(s) and telephone number(s) of the person(s) who may be contacted and/or take action in the event that the CompMed staff notes an emergency condition with the research animals.

Thank you for your continued cooperation with the Institutional Animal Care and Use Committee.

Sincerely,

[redacted]

Institutional Animal Care and Use Committee

[redacted]

cc: [redacted]
Attending Veterinarian
Division of Comparative Medicine

[redacted]
Project Manager
Division of Comparative Medicine

[redacted]
Senior Associate Dean for Research
Institutional Official

[redacted] www.evms.edu
P.O. Box 1980 Norfolk, VA 23501-1980 Phone: [redacted] Fax: 757 [redacted]

EVMS

JUL 23 2014

July 15, 2014

Carol [redacted]
[redacted]

[redacted] Chair
Institutional Animal Care and Use Committee
Eastern Virginia Medical School
Norfolk, VA 23507

Dear [redacted]:

I would like to have the appended research protocols (briefly outlined below) amended to my approved IACUC protocols entitled "Regulation of Fetal-Placental Development in the Primate" (#12-010) and Estrogen Regulation of Insulin Signaling in the Non-Pregnant Baboon" (#12-007). *Amendment 1*: In both #12-010 and #12-007 IACUC protocols, we are approved to obtain biopsies of skeletal muscle from adolescent and adult baboons using a punch biopsy. We have found that this procedure yields non-uniform threads of muscle fibers which are almost impossible to embed in paraffin. As such we cannot perform requisite histologic and immuno-histochemical analyses thereby limiting conduct of the approved studies. Accordingly, we are requesting permission to collect muscle biopsies using a small scalpel. This would allow us to obtain a uniform sample which we can orient appropriately in paraffin to permit histology studies as well as provide sufficient tissue that can be frozen (liquid nitrogen) for subsequent biochemical analyses (e.g. Western blot; RT-PCR). *Amendment 2*: In both protocols #12-010 and #12-007, we are approved to perform intravenous glucose tolerance tests (iv GTT) but only in protocol #12-007 are we approved to obtain muscles biopsies before (time 0) and at 30 minutes after onset of iv glucose infusion. As insulin levels rise rapidly after onset of glucose infusion, the second biopsy permits analysis of response of muscle to endogenous insulin i.e. compare expression of proteins in the 0 time and 30 minute samples. Preliminary studies (previous amendment approved July, 2012) confirmed these time points are appropriate for the studies proposed. Accordingly, I am requesting that we conduct this multiple biopsy procedure during conduct of two iv GTT experiments performed in adolescent baboons first at 3-4 years of age and subsequently at 6-10 years of age.

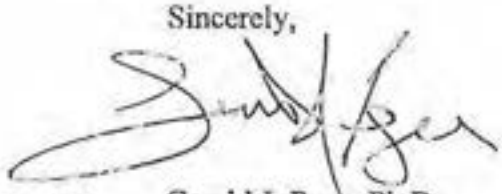
To summarize the muscle biopsy procedure, the area of skin overlying the vastus lateralis muscle is shaved and prepared using sterile procedures as outlined previously. A small incision is made through the skin with a #10 surgical blade to access the underlying muscle for biopsy. We are requesting that this incision be increased from 0.5 cm to 1 cm. A 2.5-3.0 cm (length) x 0.50- 0.75 cm (width) by 0.20-0.25 cm (depth) segment of skeletal muscle will then be surgically isolated using a #10 surgical blade. Hemostasis will be maintained with direct pressure and the muscle area packed with gel foam and the superficial incision closed with absorbable suture. For experiments in which 0 and 30 minute samples are collected, the first will be obtained from the

right (or left) limb and the second from the alternate limb. Finally, we will continue to follow the post-operative recovery/analgesia protocols as currently approved in #12-010 and #12-007.

I have also appended a summary manuscript (Patel et al, 2011; open access journal) and abstracts of experiments (Edgett et al, 2013; Cobley et al, 2014) in which comparable procedures have been used to obtain multiple muscle biopsies in human studies; biopsies up to 290 mg have been collected. Also, please note Figure 5 (Patel et al, 2011) which shows isolation of a sample of vastus lateralis that appears (on computer view) to exceed the size (dimensions) we are requesting in the current study. That manuscript also describes (Table 1) the level of pain (VAS pain score 0 = no pain; 100 = as bad as it can be) perceived during (median 7; range 1-34) and 1 and 7 days after the procedure (median 4 and 1, respectively). Complications were minimal and when reported were often wound hematomas.

The IACUC's time and effort in reviewing these amendments is most appreciated.

Sincerely,



Gerald J. Pepe, Ph.D.

REQUEST TO AMEND A PREVIOUSLY APPROVED IACUC PROTOCOL

Institutional Animal Care and Use Committee (IACUC)

JUL 23 2014

Eastern Virginia Medical School
Office of Research
IACUC Office
[redacted]
Office: 757-[redacted]
Fax: 757[redacted]

FOR OFFICE USE ONLY
Date Received: 7/23/14 [redacted] **Review Method:** X FCR / ____ FR / ____ Administrative (*Personnel Changes Only*)
IBC Approval? X Yes / ____ No **IBC Approval Date:** 04-2014 **Final Approval Date** 8/7/14 [redacted]

General Information and Instructions:

1. All requested amendments must be approved by the IACUC before they are implemented.
2. Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian before the changes are submitted to the IACUC Office.
3. A request to increase the number of animals assigned to a protocol must be approved by the IACUC before the animals are ordered.
4. Submit the original signed amendment form to the IACUC Office located in [redacted], and e-mail the MSWord version of the form to the IACUC administrator.
5. *The IACUC reserves the right to require submission of an IACUC "Initial Review Form" based upon the Committee's assessment of the amendment(s) requested herein.*

I. ADMINISTRATIVE INFORMATION

Protocol Number: 12-010	**Protocol Initial Approval Date: 7-30-2012**
Protocol Title: Regulation of Fetal-placental Development in the Primate	
Principal Investigator: Gerald J Pepe, PHD	
Approved USDA Pain Code Level(s) – *not required for personnel additions only*: **B &D**	

II. PERSONNEL

List all personnel to be added to or removed from the protocol. All persons authorized to work on the protocol must have completed the required CITI/LATA and OHSP training modules and must have completed a health/risk assessment with the Occupational Health Department.

Name	Added (+) or Removed (-)	E-mail Address *(additions only)*	CITI/LATA Training Certification Number *(additions only)*	CITI/LATA Training Species Module(s) Completed *(additions only)*	OHSP Training Certification Number *(additions only)*	Occupational Health/ Risk Assessment Date *(additions only)*
1.						
2.						
3.						
Procedure(s) to be performed by personnel addition #1:						
Procedure(s) to be performed by personnel addition #2:						
Procedure(s) to be performed by personnel addition #3:						

III. PROPOSED CHANGES TO THE ORIGINALLY APPROVED PROTOCOL

☐ **A. CHANGE OF SPONSOR/FUNDING SOURCE**

List the new/approved funding source, the project/grant number, the project/grant title (if different from the IACUC protocol title), and the award period.

☐ **B. CHANGE IN PROJECT SITE(S)**

List all new locations (i.e., building and room number(s)) and indicate what procedure(s) will be performed in each new location.

☐ **C. CHANGE OF SPECIES AND/OR STRAIN**

List the species to be added to or removed from the protocol. Please provide the rationale for any species/strain additions and indicate the USDA Pain Category assignment for each new species/strain. Also, list any anticipated changes in veterinary care and/or husbandry due to the new species/strain.

Species and/or strain	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E

☐ **D. CHANGE IN ANIMAL NUMBERS**

List the number of animals to be added to or removed from the protocol. If animals will be added, please provide the justification for the increase and indicate the number of animals to be assigned to each USDA Pain Category.

Species and/or strain	Year (1, 2 or 3)	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E	TOTAL

Will the animals be ordered through CompMed? _____Yes _____ No ***(Identify the source and provide the rationale/justification below.)***

Will the animals require special housing (e.g., specific bedding requirements, isolator cages, special feed, etc.)
_____ Yes ***(Please specify below.)*** _____ No

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

[X] **E. CHANGE IN EXPERIMENTAL PROCEDURES AND/OR INSTRUMENTATION**

Describe, in detail, the proposed change(s). *Please indicate potentially painful/distressing effects on the animals as a result of the proposed change(s). Also, please indicate any expected adverse effects and any changes in monitoring, husbandry, housing, early endpoint, and/or experimental endpoint. Finally, please complete the checklist below.*

Amendment 1: We would like to add skeletal muscle biopsy to our IVGT test currently approved in 12-010. Samples would be taken at time 0 and 30 respectively from alternating legs. Animals would undergo the biopsy twice (2) during the study, pre-pubertal 3-4years of age and post puberty 6-10years.

Amendment 2: We would like to obtain skeletal muscle samples using a scalpel blade rather than the currently approved punch biopsy. The skin area is shaved and sterilized as currently approved. A small incision (0.5cm – 1cm) is made exposing the vastus lateralis. At time '0' and '30', a 2.5-3.0cm(L) x 0.5-0.75cm(W) x 0.2-0.25(D) segment of muscle is surgically removed from alternating legs. The area is packed with gel foam to minimize bleeding. The skin is then closed with absorbable suture.

Post-op analgesia (Banamime) would be given at completion of the glucose test and then 2days BID as currently approved. Animals will be monitored for appetite, fecal output and overall change in behavior. The veterinarian will be contacted if pain medication is needed beyond the 2days post procedure.

Please check all that apply to the proposed change(s). Complete the required IACUC Attachment and submit it along with the amendment form.	
Biohazardous Agents: ____ Yes __X__ No (e.g., recombinant DNA, RNA, all tissue or cell samples, laboratory-induced infection, cultured pathogens)	Complete and submit **Attachment D**
Radioisotopes, *in vivo*: ____ Yes __X__ No	Complete and submit **Attachment D**
Chemical Agents: ____ Yes __X__ No (e.g., known or suspected chemical hazards, mutagens, or teratogens)	Complete and submit **Attachment D**
Stress or Prolonged Restraint: ____ Yes __X__ No	Complete and submit **Attachment C**
Food and/or Water Deprivation: ____ Yes __X__ No	Complete and submit **Attachment C**
Surgical Procedures: ____ Yes __X_ No (i.e., single survival, multiple survival, or non-survival)	Complete and submit **Attachment E**
Antibody Production: ____ Yes __X__ No	Complete and submit **Attachment F**
Toxicity Testing (LD50): ____ Yes __X__ No	Complete and submit **Attachment G**
Lasers or Penetrating Electromagnetic Radiation: ____ Yes __X__ No	
Collection of Tissues, Cells, or Organs: _X___ Yes ____ No (Please explain below.) Skeletal Muscle will be obtained during	

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

the experiment:	
Tumor Transplantation or Induction: ____ Yes __X__ No	
Special Diet: ____ Yes __X__ No (Please explain below.)	
Other : N/A (Please specify below.)	

☐ F. CHANGE IN ANESTHESIA, THERAPEUTICS, AND/OR EXPERIMENTAL AGENTS

	Dose (mg/kg)	Route of Administration	Frequency of Administration	
Sedatives/Tranquilizers				
Anesthetics - General				
Anesthetics - Local				
				Length of Administration
Analgesics				
Flunixin meglumine (Banamine)	**2mg / kg**	**IM**	**Completion of glucose test and 2days BID**	**3days total (PRN with consult from vet)**
Antibiotics				
Miscellaneous				

EXPERIMENTAL AGENTS:

Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _____

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

INVESTIGATOR ASSURANCES:	
X	*I understand that it is my responsibility as the Principal Investigator to ensure that all personnel who work on this protocol are adequately trained. That training may be provided by me, an experienced member of the investigative staff, the Attending Veterinarian, a qualified Division of Comparative Medicine (CompMed) staff member, or an appropriate outside source. I hereby affirm that each person added to this protocol by way of this amendment request has been adequately trained to perform the procedure(s) indicated above. Any person who has not been adequately trained will be so trained prior to performing the procedure(s).*
X	*No animal involved in this project will be subjected to discomfort, pain, or distress, unless it is unavoidable in the conduct of scientifically valuable research and approval of such has been approved by the IACUC.*
X	*I agree to comply with all federal and institutional policies governing the use of animals used in this project.*

PRINCIPAL INVESTIGATOR:	
Printed Name: Gerald J. Pepe, PH.D.	
Signature:	**Date:** 7/15/2014

APPROVAL SIGNATURES:	
VETERINARY PRE-REVIEW: *Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian before the changes are submitted to the IACUC for review.*	
Attending Veterinarian Printed Name: [redacted]	
Attending Veterinarian Signature:	**Date:**
FINAL IACUC APPROVAL: *All revisions must be approved by the IACUC prior to implementation.*	
IACUC Chair or Vice Chair Printed Name: [redacted]	8-12-2014
IACUC Chair or Vice Chair Signature:	**Date:**

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

EVMS
Eastern Virginia Medical School

November 2, 2017

Gerald J. Pepe, Ph.D.
Chair, Department of Physiological Sciences
Eastern Virginia Medical School
[redacted]
Norfolk, Virginia 23507

Dear Dr. Pepe:

The Institutional Animal Care and Use Committee reviewed your request to amend the protocol entitled, ***Regulation of Fetal-placental Development in the Primate*** **(IACUC #15-009)**, at its November 2, 2017 meeting. **The following amendments to the protocol were approved:**

1. **addition of [redacted] to insert and remove the pessary device developed by CONRAD, and**
2. **addition of the following 3 studies to ascertain the potential for progesterone delivered directly to the cervical region via a silicone pessary to delay parturition and thus prevent premature birth: (1) Control Study; (2) Pessary Device Study; and (3) Intramuscular (IM) Injection Study. The studies will be performed in six (6) non-pregnant regularly menstruating baboons.**

Although the Division of Comparative Medicine (CompMed) will make every effort to provide housing, husbandry, and veterinary support for your research project, IACUC approval of this amendment request does not guarantee that CompMed will be able to accommodate the work immediately. Available space and resources within the CompMed animal facility are determined by the number of ongoing projects and the animal census at any given time. Please consult with the CompMed management team to confirm availability prior to implementing the approved modification(s).

It is the responsibility of the Principal Investigator to notify the Committee, in writing, of any deviation from the IACUC-approved protocol. Please note that a change in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian prior to submitting the amendment request to the IACUC for approval.

It is also the responsibility of the Principal Investigator to provide for regular observation of the health of the research animals and to provide the Division of Comparative Medicine (CompMed) with the name(s) and telephone number(s) of the person(s) who may be contacted and/or take action in the event that the CompMed staff notes an emergency condition with the research animals.

Thank you for your continued cooperation with the Institutional Animal Care and Use Committee.

Office of Research
[redacted]
NORFOLK, VA 23507
p: 757[redacted]
f: 757[redacted]
www.evms.edu

Sincerely,

[redacted], Chair
Institutional Animal Care and Use Committee

[redacted]

cc: [redacted]
Attending Veterinarian
Division of Comparative Medicine

[redacted]
Program Manager
Division of Comparative Medicine

[redacted]
Senior Associate Dean for Research
Institutional Official

EVMS
Eastern Virginia Medical School

September 8, 2017

RECEIVED
SEP 29 RECD
9/29/17 OK
OFFICE OF RESEARCH
EVMS

[redacted] Chair
Institutional Animal Care and Use Committee
Eastern Virginia Medical School
Norfolk, VA 23507

Dear [redacted]:

I would like to have the appended research protocol amended to my approved IACUC protocol entitled "Regulation of Fetal-Placental Development in the Primate" (#15-009). The amendment is described in the attached IACUC forms and outlined below. Basically, we have developed a collaboration with [redacted] and CONRAD to utilize the baboon as an experimental translational model to develop a new clinical paradigm to ascertain the potential for progesterone delivered directly to the cervical region via a silicone pessary to delay parturition and thus prevent premature birth, a major problem that is associated with significant fetal/neonatal morbidity and mortality particularly in less developed countries. Currently, progesterone therapy, which actually is an intramuscular (im) injection of 17-hydroxyprogesterone starting at about 50%-60% of gestation has been shown to delay premature uterine contractions. However, to be effective the 17-hydroxyprogesterone must be given once every week for up to 8 weeks or longer depending on when premature contractions are first initiated. Unfortunately, for almost all women in less developed countries, making one trip to a physician for an injection is challenging but doable but multiple return visits are impossible. Therefore, providing progesterone via a vaginal device that allows sustained release and formation of 17-hydroxyprogesterone locally over an 8-12 week period would be most desirable. The cervical pessary has a long history of use to reduce the risk of preterm birth through mechanical and physical mechanisms. However, sustained vaginal release of progesterone from a pessary, along with the benefits of the device itself on inhibiting premature uterine contraction, has never been tested. Before proceeding to human studies, a progestin pessary that provides sustained levels of progesterone/17-hydroxprogesterone needs to be developed and tested in an appropriate animal model. Our laboratory has for many years studied the role of estrogen in primate pregnancy and established the baboon as a translational model for studies of human pregnancy. Thus, the baboon is an ideal animal model to ascertain the clinical potential of a progestin-eluting pessary. However, before requesting that a pessary be placed in pregnant animals, it is first necessary to ascertain whether the pessary will provide a level of progesterone/17-hydroxyprogesterone comparable to that achieved by im injection of 17-hydroxprogesterone in nonpregnant animals and thus in the absence of pregnancy-induced changes in maternal physiology (e.g. cardiac output, uteroplacental blood flow). Therefore, we are requesting permission to use 6 nonpregnant, regularly menstruating female

[redacted]

baboons currently in our EVMS colony and perform 3 studies: [1] in a control menstrual cycle, obtain peripheral (saphenous vein) blood samples (3 ml), a swab of the cervix and conduct a noninvasive 2D ultrasound of the uterus to determine endometrial growth/thickness in ketamine/isoflurane (nose cone) anesthetized animals in the early follicular, late follicular, and early luteal phases and a blood sample, a cervical swab, a cervical biopsy and a noninvasive 2D ultrasound of the uterus in the late luteal phase under ketamine:propofol or ketamine/isoflurane (nose cone) anesthesia. [2] Following a one month recovery, in the subsequent menstrual cycle, we propose to insert after completion of menses under ketamine/isoflurane (nose cone) anesthesia a specially designed pessary containing 90-120 mg progesterone into the vagina using a vaginal speculum and obtain maternal peripheral blood samples (3 ml) and a cervical swab and a 2D ultrasound of the uterus under ketamine/isoflurane sedation at 3-5 (early follicular), 10-12 (late follicular), and 16-18 (early luteal) and a blood sample, cervical swab and a cervical biopsy and 2D ultrasound 24-26 days after insertion of the pessary. The pessary will also be removed at this point. [3] After a one month period, the blood sampling/uterine/cervical protocols will be performed in these same baboons injected im with 17-hydroxyprogesterone (ketamine sedation) on days 2 and 7 after menstruation.

The levels of progesterone and 17-hydroxyprogesterone in blood samples, cervical biopsy and swabbed cells will be determined by RIA to assess release, metabolism and uptake of progesterone from the pessary at the site of insertion and secretion into the peripheral blood. The estimation of uterine endometrial thickness will show the extent to which progesterone was delivered to the uterus as endometrial thickness is a well- established response to progesterone. Thus, these noninvasive experiments will provide the requisite preliminary data for potential therapeutic application of a progesterone pessary in pregnancy. The pessary developed by CONRAD is as small as possible and still contain a sufficient amount of dissolved progesterone (17-hydroxyprogesterone not soluble) and of chemical composition to permit sustained/constant release and yet is relatively soft and pliable. Thus, by design the pessary is likely to conform to the size and shape of the baboon vagina and not induce any local inflammation or tissue damage and release a constant amount of progesterone over the study period. Moreover, using a vaginal speculum will facilitate placing the device close to the cervix. A description of the pessary is outlined in the IACUC protocol and a prototype available to the IACUC for examination. [redacted] who places pessary devices in women will insert the device in baboons as she has all of the requisite experience and expertise.

The IACUC's time and effort in reviewing this amendment is most appreciated.

Sincerely,

Gerald J. Pepe, Ph.D.
Professor and Chair

GJP[redacted]

April 2010
Updated February 2011; March 2011; April 2012; August 2013

RECEIVED
9/29/17 CSK
SEP 29 RECD

REQUEST TO AMEND A PREVIOUSLY APPROVED IACUC PROTOCOL

Institutional Animal Care and Use Committee (IACUC)

Eastern Virginia Medical School
Office of Research

IACUC Office
[redacted]

Office: 757-4[redacted]
Fax: 757-[redacted]

FOR OFFICE USE ONLY
Date Received: 9/29/17 [redacted] **Review Method:** X FCR / ___ FR / ___ Administrative (*Personnel Changes Only*)
IBC Approval? X Yes / ___ No **IBC Approval Date:** 9/15/15 **Final Approval Date:** 11/2/17 [redacted]

General Information and Instructions:

1. All requested amendments must be approved by the IACUC **before** they are implemented.
2. Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian **before** the changes are submitted to the IACUC Office.
3. A request to increase the number of animals assigned to a protocol must be approved by the IACUC **before** the animals are ordered.
4. **Submit the original signed amendment form to the IACUC Office located in [redacted], and e-mail the MSWord version of the form to the IACUC Administrator.**
5. ***The IACUC reserves the right to require submission of an IACUC "Initial Review Form" based upon the Committee's assessment of the amendment(s) requested herein.***

I. ADMINISTRATIVE INFORMATION

Protocol Number: 15-009	**Protocol Initial Approval Date: 07/9/15**
Protocol Title: Regulation of Fetal-Placental Development in the Primate	
Principal Investigator: Gerald J Pepe, Ph.D.	
Approved USDA Pain Code Level(s) – *not required for personnel additions only*: **B, C, and D**	

II. PERSONNEL

List all personnel to be added to or removed from the protocol. All persons authorized to work on the protocol must have completed the required CITI/LATA and OHSP training modules and must have completed a health/risk assessment with the Occupational Health Department.

Name	Added (+) or Removed (-)	E-mail Address *(additions only)*	CITI/LATA Training Certification Number *(additions only)*	CITI/LATA Training Species Module(s) Completed *(additions only)*	OHSP Training Certification Number *(additions only)*	Occupational Health/ Risk Assessment Date *(additions only)*
[redacted]	+	[redacted]	[redacted]	NHP	[redacted]	5/24/17
2.						
3.						
Procedure(s) to be performed by personnel addition #1: Insertion and removal of pessary device						
Procedure(s) to be performed by personnel addition #2:						
Procedure(s) to be performed by personnel addition #3:						

III. PROPOSED CHANGES TO THE ORIGINALLY APPROVED PROTOCOL

☐ **A. CHANGE OF SPONSOR/FUNDING SOURCE**

List the new/approved funding source, the project/grant number, the project/grant title (if different from the IACUC protocol title), and the award period.

☐ **B. CHANGE IN PROJECT SITE(S)**

List all new locations (i.e., building and room number(s)) and indicate what procedure(s) will be performed in each new location.

☐ **C. CHANGE OF SPECIES AND/OR STRAIN**

List the species to be added to or removed from the protocol. Please provide the rationale for any species/strain additions and indicate the USDA Pain Category assignment for each new species/strain. Also, list any anticipated changes in veterinary care and/or husbandry due to the new species/strain.

Species and/or strain	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E

☐ **D. CHANGE IN ANIMAL NUMBERS**

List the number of animals to be added to or removed from the protocol. If animals will be added, please provide the justification for the increase and indicate the number of animals to be assigned to each USDA Pain Category.

Species and/or strain	Year (1, 2 or 3)	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E	TOTAL

Will the animals be ordered through CompMed? _____Yes _____ No ***(Identify the source and provide the rationale/justification below.)***

Will the animals require special housing (e.g., specific bedding requirements, isolator cages, special feed, etc.)

_____ Yes ***(Please specify below.)*** _____ No

USDA Pain Code B – Breeding or holding colony; no animal manipulation.
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USDA Pain Code E – Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

[X] **E. CHANGE IN EXPERIMENTAL PROCEDURES AND/OR INSTRUMENTATION**

Describe, in detail, the proposed change(s). *Please indicate potentially painful/distressing effects on the animals as a result of the proposed change(s). Also, please indicate any expected adverse effects and any changes in monitoring, husbandry, housing, early endpoint, and/or experimental endpoint. Finally, please complete the checklist below.*

Our laboratory has for many years studied the role of estrogen in primate pregnancy and established the baboon as a translational model for studies of human pregnancy. Thus, the baboon is an ideal animal model to ascertain the clinical potential of a progestin-eluting pessary. However, before requesting that a pessary be placed in pregnant animals, it is first necessary to ascertain whether the pessary will provide a level of progesterone/17-hydroxyprogesterone comparable to that achieved by intramuscular (IM) injection of 17-hydroxprogesterone in nonpregnant animals and thus in the absence of pregnancy-induced changes in maternal physiology (e.g. cardiac output, uteroplacental blood flow).

We would like to use 6 nonpregnant regularly menstruating female baboons from the current colony and perform 3 studies: **[1]** in a control menstrual cycle, obtain peripheral (saphenous vein) blood samples (3 ml; 1 sample per phase for a total of 4 samples per cycle and 12 ml per cycle), a swab of the cervix, and conduct a noninvasive 2D ultrasound of the uterus to determine endometrial growth/thickness under ketamine/propofol or ketamine/isoflurane anesthesia as currently approved under protocol 15-009. This will be done at the early follicular, late follicular and early and late luteal phases of the cycle. **[2]** Following a one month recovery period, under approved sedation we will insert a specially designed pessary (4.5 cm in diameter by 1.5 cm) containing 90-120 mg progesterone into the vagina using a vaginal speculum and obtain maternal peripheral blood samples (3 ml) and a cervical swab and conduct a 2D ultrasound of the uterus. On days 3-5 (early follicular), 10-12 (late follicular), 16-18 (early luteal), and 24-26 (late luteal) days after insertion of the pessary, under approved sedation, we will obtain 3 ml blood sample (1 sample per phase for a total of 4 samples per cycle and 12 ml per cycle), vaginal swab, and ultrasound under approved sedation. The pessary will be removed at approximately 24-26 days following placement under approved sedation using a vaginal speculum. Animals will be monitored daily following the placement of the device for any indication of spontaneous removal of device or distress or pain. No pain is expected but if necessary, Ketoprofen IM or PO will be given PRN as approved in the current protocol. **[3]** After a one month period, the blood sampling/uterine/cervical protocols will be performed in these same baboons injected IM with 17-hydroxyprogesterone (ketamine sedation) on days 2 and 7 after menstruation.

Please check all that apply to the proposed change(s). Complete the required IACUC Attachment and submit it along with the amendment form.	
Biohazardous Agents: ____ Yes __x__ No (e.g., recombinant DNA, RNA, all tissue or cell samples, laboratory-induced infection, cultured pathogens)	Complete and submit **Attachment D**
Radioisotopes, *in vivo*: ____ Yes _x___ No	Complete and submit **Attachment D**

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Chemical Agents: ____ **Yes** __x__ **No** (e.g., known or suspected chemical hazards, mutagens, or teratogens)	Complete and submit **Attachment D**
Stress or Prolonged Restraint: ____ **Yes** __x__ **No**	Complete and submit **Attachment C**
Food and/or Water Deprivation: ____ **Yes** ___x_ **No**	Complete and submit **Attachment C**
Surgical Procedures: ____ **Yes** __x__ **No** (i.e., single survival, multiple survival, or non-survival)	Complete and submit **Attachment E**
Antibody Production: ____ **Yes** __x__ **No**	Complete and submit **Attachment F**
Toxicity Testing (LD50): ____ **Yes** _x___ **No**	Complete and submit **Attachment G**
Lasers or Penetrating Electromagnetic Radiation: ____ **Yes** __x__ **No**	
Collection of Tissues, Cells, or Organs: __x__ **Yes** ____ **No** (Please explain below.) Blood samples (four 3 ml samples per cycle for a total of 12 ml per cycle) and cervical swabs	
Tumor Transplantation or Induction: ____ **Yes** __x__ **No**	
Special Diet: ____ **Yes** ___x_ **No** (Please explain below.)	
Other : N/A (Please specify below.)	

[X] **F. CHANGE IN ANESTHESIA, THERAPEUTICS, AND/OR EXPERIMENTAL AGENTS**

	Dose (mg/kg)	Route of Administration	Frequency of Administration	
Sedatives/Tranquilizers				
Anesthetics - General				
Anesthetics - Local				
				Length of Administration
Analgesics				

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USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

Antibiotics				
Miscellaneous				

EXPERIMENTAL AGENTS:

Agent: _Progesterone 4.1%; 5.5% silicone oil w/w **Agent Vehicle:** _Silicone pessary device_____
Route/Site: _____Intravaginal_____ **Volume per administration:** ___5.5 mg/day ___
Frequency of administration: _____continual from day ~2 of menstrual cycle to ~d21-28_____
Expected side effects and/or changes in animal behavior: __Possible interruption of mense. Possible GI upset. If decreased appetite, the diet will be modified under consultation with the Attending Veterinarian.
—

Agent: _____Hydroxyprogesterone caproate __ **Agent Vehicle:** ___Castor oil _____
Route/Site: _Intramuscular injection (IM)_____ **Volume per administration:** __250mg/mL_(1ml)___
Frequency of administration: _____2x on day 2 and day 7 after menstruation_____
Expected side effects and/or changes in animal behavior: ___Possible interruption of mense. Possible GI upset. If decreased appetite, the diet will be modified under consult with the Attending Veterinarian. Possible tenderness at the injection site_____

Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _____
_____

INVESTIGATOR ASSURANCES:

X	*I understand that it is my responsibility as the Principal Investigator to ensure that all personnel who work on this protocol are adequately trained. That training may be provided by me, an experienced member of the investigative staff, the Attending Veterinarian, a qualified Division of Comparative Medicine (CompMed) staff member, or an appropriate outside source. I hereby affirm that each person added to this protocol by way of this amendment request has been adequately trained to perform the procedure(s) indicated above. Any person who has not been adequately trained will be so trained prior to performing the procedure(s).*
X	*No animal involved in this project will be subjected to discomfort, pain, or distress, unless it is unavoidable in the conduct of scientifically valuable research and approval of such has been approved by the IACUC.*
X	*I agree to comply with all federal and institutional policies governing the use of animals used in this project.*

USDA Pain Code B – Breeding or holding colony; no animal manipulation.
USDA Pain Code C – Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D – Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E – Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

Agent: Hydroxyprogesterone caproate **Agent Vehicle:** castor oil
Route/Site: IM **Volume per administration:** 250mg/mL (1ml)
Frequency of administration: 2x day 2 and 7 after menstruation
Expected side effects and/or changes in animal behavior: Possible interruption of mense. Possible GI upset. If decreased appetite, the diet will be modified under consult with the attending Possible tenderness at the injection site

Agent: **Agent Vehicle:**
Route/Site: **Volume per administration:**
Frequency of administration:
Expected side effects and/or changes in animal behavior:

INVESTIGATOR ASSURANCES:

☒	*I understand that it is my responsibility as the Principal Investigator to ensure that all personnel who work on this protocol are adequately trained. That training may be provided by me, an experienced member of the investigative staff, the Attending Veterinarian, a qualified Division of Comparative Medicine (CompMed) staff member, or an appropriate outside source. I hereby affirm that each person added to this protocol by way of this amendment request has been adequately trained to perform the procedure(s) indicated above. Any person who has not been adequately trained will be so trained prior to performing the procedure(s).*
☒	*No animal involved in this project will be subjected to discomfort, pain, or distress, unless it is unavoidable in the conduct of scientifically valuable research and approval of such has been approved by the IACUC.*
☒	*I agree to comply with all federal and institutional policies governing the use of animals used in this project.*

PRINCIPAL INVESTIGATOR:

Printed Name: Gerald J. Pepe, Ph.D.

Signature: **Date:** September 28, 2017

USDA Pain Code B = Breeding or holding colony, no animal manipulation
USDA Pain Code C = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.
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USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian before the changes are submitted to the IACUC for review. **Attending Veterinarian Printed Name:** [redacted] **Attending Veterinarian Signature:** [redacted]	**Date:** 9/25/17
FINAL IACUC APPROVAL: *All revisions must be approved by the IACUC prior to implementation.* **IACUC Chair or Vice Chair Printed Name:** [redacted] **IACUC Chair or Vice Chair Signature:** [redacted]	Nov 7 2017 **Date:**

***USDA Pain Code B** = Breeding or holding colony, no animal manipulation.*
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***USDA Pain Code E** = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.*

EVMS
Eastern Virginia Medical School

May 3, 2018

Gerald J. Pepe, Ph.D.
Chair, Department of Physiological Sciences
Eastern Virginia Medical School
[redacted]
Norfolk, Virginia 23507

Dear Dr. Pepe:

The Institutional Animal Care and Use Committee reviewed your request to amend the protocol entitled, ***Regulation of Fetal-placental Development in the Primate*** **(IACUC #15-009)**, at its May 3, 2018 meeting. **The following amendment to the protocol was approved:**

> **Permission to collect one ectocervical (vaginal/cervical) biopsy in non-pregnant female animals during the late luteal phase of the menstrual cycle under the following conditions for a total of 3 biopsies: (1) during use of the pessary device; (2) after the 17-hydroxyprogesterone (17-OHP) injection; and (3) in the recovery period after the 17-OHP injection. It is the understanding of the Committee that the biopsies will be performed on sedated and anesthetized animals by [redacted], and the animals will be monitored for bleeding and/or signs of pain for 5 days post-procedurally.**

Although the Division of Comparative Medicine (CompMed) will make every effort to provide housing, husbandry, and veterinary support for your research project, IACUC approval of this amendment request does not guarantee that CompMed will be able to accommodate the work immediately. Available space and resources within the CompMed animal facility are determined by the number of ongoing projects and the animal census at any given time. Please consult with the CompMed management team to confirm availability prior to implementing the approved modification(s).

It is the responsibility of the Principal Investigator to notify the Committee, in writing, of any deviation from the IACUC-approved protocol. Please note that a change in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian prior to submitting the amendment request to the IACUC for approval.

It is also the responsibility of the Principal Investigator to provide for regular observation of the health of the research animals and to provide the Division of Comparative Medicine (CompMed) with the name(s) and telephone number(s) of the person(s) who may be contacted and/or take action in the event that the CompMed staff notes an emergency condition with the research animals.

Thank you for your continued cooperation with the Institutional Animal Care and Use Committee.

Office of Research
[redacted]
NORFOLK, VA 23507
ph 757[redacted]
fx 757[redacted]
www.evms.edu

Sincerely,

[redacted] Chair
Institutional Animal Care and Use Committee

[redacted]

cc: [redacted]
Attending Veterinarian
Division of Comparative Medicine

[redacted]
Program Manager
Division of Comparative Medicine

[redacted]
Senior Associate Dean for Research
Institutional Official

EVMS
Eastern Virginia Medical School

April 16, 2018

RECEIVED
APR 17 2018
OFFICE FOR RESEARCH
EVMS

[redacted] Chair
Institutional Animal Care and Use Committee
Eastern Virginia Medical School
Norfolk, VA 23507

Dear [redacted]:

I would like to have the appended research protocol amended to my approved IACUC protocol entitled "Regulation of Fetal-Placental Development in the Primate" (#15-009). The amendment is described in the attached IACUC forms and outlined below. Briefly, in collaboration with [redacted] and the CONRAD Program, we have been approved to use 6 nonpregnant, regularly menstruating female baboons currently in our EVMS colony and perform the following 3 studies: [1] in a control menstrual cycle, obtain peripheral (saphenous vein) blood samples (3 ml), a swab of the cervix and conduct a noninvasive 2D ultrasound of the uterus to determine endometrial growth/thickness under currently approved 15-009 sedation protocol in the early follicular, late follicular, early luteal and late luteal phases; [2] Following a one month recovery, in the subsequent menstrual cycle, insert after completion of menses under sedation a specially designed pessary containing progesterone into the vagina using a vaginal speculum and obtain maternal peripheral blood samples (3 ml) and a cervical swab and a 2D ultrasound of the uterus under ketamine/isoflurane sedation at 3-5 (early follicular), 10-12 (late follicular), 16-18 (early luteal) and 24-26 (late luteal) days after insertion of the pessary which will also be removed at this point; [3] After a one month period, the blood sampling/ uterine/cervical protocols will be performed in these same baboons injected IM with 17-hydroxyprogesterone (ketamine sedation) on days 2 and 7 after menstruation. The levels of progesterone and 17-hydroxyprogesterone in blood samples-and swabbed cervical cells will be determined by RIA to assess release, metabolism and uptake of progesterone from the pessary at the site of insertion and secretion into the peripheral blood.

While obtaining cervical cells via swab is very useful, it is apparent that we are only obtaining epithelial cells lining the surface and thus it is not known whether the cervical cells below the surface and which are critically important during pregnancy are also taking up the progesterone and exhibiting progesterone induced changes in the expression of key proteins and/or mRNA. Accordingly, it is important to obtain and thus we are requesting permission to collect one ectocervical biopsy in the late luteal phase during pessary use, in the late luteal phase after 17OH P injection, and in the late luteal phase in the recovery period after the 17 OH P injection, i.e. the 3 groups of animals in the approved protocol. The animals would be sedated as approved and

Physiological Sciences
[redacted]
P.O. BOX 1980
NORFOLK, VA 23501-1980
[redacted]
www.evms.edu

anesthetized as approved. Briefly, the perineal area is prepped with antiseptic solution and using a speculum, the proposed biopsy area cleaned with saline. Once a clear field is achieved, a 3 x 5 mm biopsy (approximately 20 mg tissue) approximately 2/3 of the distance from the posterior fornix to the introitus is obtained using Tischler forceps. Caution will be taken to assure that the serosal region is not impacted and thus the biopsy is not very deep into the cervix. Hemostasis will be attempted via application of adequate pressure, whenever possible and treated with Gel Foam or Monsel's solution if necessary. One dose of approved Banamine will be administered following the biopsies, then PRN based on consultation with the Attending Veterinarian and animals monitored for bleeding or signs of pain for 5 days. A description of the procedure is outlined in the IACUC protocol and will be performed by [redacted] who has performed the same procedure in women and has the requisite experience and expertise.

The IACUC's time and effort in reviewing this amendment is most appreciated.

Sincerely,

Gerald J. Pepe, Ph.D.
Professor and Chair

GJP/[redacted]

REQUEST TO AMEND A PREVIOUSLY APPROVED IACUC PROTOCOL

Institutional Animal Care and Use Committee (IACUC)

Eastern Virginia Medical School
Office of Research

IACUC Office
[redacted]

Office: 757-[redacted]
Fax: 757-[redacted]

FOR OFFICE USE ONLY
Date Received: 4/17/18 [redacted] **Review Method:** X FCR / ___ FR / ___ Administrative (*Personnel Changes Only*)
IBC Approval? X Yes / ___ No **IBC Approval Date:** 7/1/15 **Final Approval Date:** 5/3/18 [redacted]

General Information and Instructions:

1. All requested amendments must be approved by the IACUC **before** they are implemented.
2. Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian **before** the changes are submitted to the IACUC Office.
3. A request to increase the number of animals assigned to a protocol must be approved by the IACUC **before** the animals are ordered.
4. **Submit the original signed amendment form to the IACUC Office located in [redacted], and e-mail the MSWord version of the form to the IACUC Administrator.**
5. ***The IACUC reserves the right to require submission of an IACUC "Initial Review Form" based upon the Committee's assessment of the amendment(s) requested herein.***

I. ADMINISTRATIVE INFORMATION

Protocol Number: 15-009	**Protocol Initial Approval Date:** July 9, 2015
Protocol Title: Regulation of Fetal-Placental Development in the Primate	
Principal Investigator: Gerald J Pepe, Ph.D.	
Approved USDA Pain Code Level(s) – *not required for personnel additions only*: **B, C and D**	

II. PERSONNEL

List all personnel to be added to or removed from the protocol. ***All persons authorized to work on the protocol must have completed the required CITI/LATA and OHSP training modules and must have completed a health/risk assessment with the Occupational Health Department.***

Name	Added (+) or Removed (-)	E-mail Address *(additions only)*	CITI/LATA Training Certification Number *(additions only)*	CITI/LATA Training Species Module(s) Completed *(additions only)*	OHSP Training Certification Number *(additions only)*	Occupational Health/ Risk Assessment Date *(additions only)*
1.						
2.						
3.						
Procedure(s) to be performed by personnel addition #1:						
Procedure(s) to be performed by personnel addition #2:						
Procedure(s) to be performed by personnel addition #3:						

III. PROPOSED CHANGES TO THE ORIGINALLY APPROVED PROTOCOL

☐ **A. CHANGE OF SPONSOR/FUNDING SOURCE**

List the new/approved funding source, the project/grant number, the project/grant title (if different from the IACUC protocol title), and the award period.

☐ **B. CHANGE IN PROJECT SITE(S)**

List all new locations (i.e., building and room number(s)) and indicate what procedure(s) will be performed in each new location.

☐ **C. CHANGE OF SPECIES AND/OR STRAIN**

List the species to be added to or removed from the protocol. Please provide the rationale for any species/strain additions and indicate the USDA Pain Category assignment for each new species/strain. Also, list any anticipated changes in veterinary care and/or husbandry due to the new species/strain.

Species and/or strain	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E

☐ **D. CHANGE IN ANIMAL NUMBERS**

List the number of animals to be added to or removed from the protocol. If animals will be added, please provide the justification for the increase and indicate the number of animals to be assigned to each USDA Pain Category.

Species and/or strain	Year (1, 2 or 3)	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E	TOTAL

Will the animals be ordered through CompMed? _____Yes _____ No ***(Identify the source and provide the rationale/justification below.)***

Will the animals require special housing (e.g., specific bedding requirements, isolator cages, special feed, etc.)

_____ Yes ***(Please specify below.)*** _____ No

USDA Pain Code B = *Breeding or holding colony; no animal manipulation.*
USDA Pain Code C = *Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.*
USDA Pain Code D = *Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.*
USDA Pain Code E = *Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.*

[X] **E. CHANGE IN EXPERIMENTAL PROCEDURES AND/OR INSTRUMENTATION**

Describe, in detail, the proposed change(s). *Please indicate potentially painful/distressing effects on the animals as a result of the proposed change(s). Also, please indicate any expected adverse effects and any changes in monitoring, husbandry, housing, early endpoint, and/or experimental endpoint. Finally, please complete the checklist below.*

We would like to add 3 vaginal/cervical biopsies to our currently approved pessary study using non-pregnant female baboons. The animal is sedated and anesthetized prior to the procedure as outlined in the currently approved protocol. The perineal area is prepped with antiseptic solution. Using a speculum, the area of the planned biopsy is cleaned with saline. Once a clear field is reached, a 3mm x 5mm biopsy is obtained with a Tischler forcep. The average weight of the ectocervical biopsies is 20 mg. Caution will be taken to assure that the serosal region is not impacted, thus the biopsy will not be very deep into the cervix. Hemostasis will be attempted via application of adequate pressure, whenever possible. The biopsy site may be treated with Gel Foam or Monsel's solution to control bleeding.

One ectocervical biopsy will be collected under each of the following conditions for a total of 3 biopsies: 1) in the late luteal phase during use of the pessary device, 2) in the late luteal phase after 17-OHP (17-hydroxyprogesterone) injection, and 3) in the late luteal phase in the recovery period after the 17-OH P injection (control cycle). As outlined in the currently approved protocol, one dose of Banamine will be administered following the biopsies, then PRN based upon consultation with the Attending Veterinarian. The animal will be monitored for bleeding or signs of pain for 5 days post-procedure. The biopsy procedure will be performed by [redacted] who is authorized personnel on the protocol. [redacted] has performed the same procedure in women and has the requisite experience and expertise.

As stated in the currently approved protocol, there will be a wash out period between the three experimental treatments.

Please check all that apply to the proposed change(s). Complete the required IACUC Attachment and submit it along with the amendment form.	
Biohazardous Agents: ____ Yes __x__ No (e.g., recombinant DNA, RNA, all tissue or cell samples, laboratory-induced infection, cultured pathogens)	Complete and submit **Attachment D**
Radioisotopes, *in vivo*: ____ Yes __x__ No	Complete and submit **Attachment D**
Chemical Agents: ____ Yes __x__ No (e.g., known or suspected chemical hazards, mutagens, or teratogens)	Complete and submit **Attachment D**
Stress or Prolonged Restraint: ____ Yes __x__ No	Complete and submit **Attachment C**
Food and/or Water Deprivation: ____ Yes __x__ No	Complete and submit **Attachment C**
Surgical Procedures: ____ Yes __x__ No (i.e., single survival, multiple survival, or non-survival)	Complete and submit **Attachment E**
Antibody Production: ____ Yes __x__ No	Complete and submit **Attachment F**
Toxicity Testing (LD50): ____ Yes __x__ No	Complete and submit **Attachment G**

***USDA Pain Code B** = Breeding or holding colony; no animal manipulation.*
***USDA Pain Code C** = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.*
***USDA Pain Code D** = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.*
***USDA Pain Code E** = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.*

Lasers or Penetrating Electromagnetic Radiation: ____ Yes __x__ No	
Collection of Tissues, Cells, or Organs: __x__ Yes ____ No (Please explain below.) Collection of a total of 3 ectocervical (vaginal/cervical) biopsies during the late luteal phase under the following conditions: 1) during use of the pessary device, 2) after 17OH P injection, and 3) during the recovery period after 17OH P injection (i.e., one biopsy per condition)	
Tumor Transplantation or Induction: ____ Yes __x__ No	
Special Diet: ____ Yes __x__ No (Please explain below.)	
Other : N/A (Please specify below.)	

X **F. CHANGE IN ANESTHESIA, THERAPEUTICS, AND/OR EXPERIMENTAL AGENTS**

	Dose (mg/kg)	Route of Administration	Frequency of Administration	
Sedatives/Tranquilizers				
Anesthetics - General				
Anesthetics - Local				
				Length of Administration
Analgesics				
Antibiotics				
Miscellaneous				
Gel Foam	N/A	Topical/vaginal	Once at time of biopsy to help achieve hemostasis	Hours
Monsel's solution	N/A	Topical/vaginal	Once at time of biopsy to help achieve hemostasis	hours

EXPERIMENTAL AGENTS:

Agent: _____ **Agent Vehicle:** _____

Route/Site: _____ **Volume per administration:** _____

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _____
_____

Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _____
_____

INVESTIGATOR ASSURANCES:

[x]	*I understand that it is my responsibility as the Principal Investigator to ensure that all personnel who work on this protocol are adequately trained. That training may be provided by me, an experienced member of the investigative staff, the Attending Veterinarian, a qualified Division of Comparative Medicine (CompMed) staff member, or an appropriate outside source. I hereby affirm that each person added to this protocol by way of this amendment request has been adequately trained to perform the procedure(s) indicated above. Any person who has not been adequately trained will be so trained prior to performing the procedure(s).*
[x]	*No animal involved in this project will be subjected to discomfort, pain, or distress, unless it is unavoidable in the conduct of scientifically valuable research and approval of such has been approved by the IACUC.*
[x]	*I agree to comply with all federal and institutional policies governing the use of animals used in this project.*

PRINCIPAL INVESTIGATOR:

Printed Name: Gerald J Pepe, PhD

Signature: _____ **Date:** 4/16/2018

APPROVAL SIGNATURES:

VETERINARY PRE-REVIEW: *Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian before the changes are submitted to the IACUC for review.*	

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian before the changes are submitted to the IACUC for review. **Attending Veterinarian Printed Name:** [redacted] **Attending Veterinarian Signature:** [redacted]	**Date:** 4/10/2018
FINAL IACUC APPROVAL: *All revisions must be approved by the IACUC prior to implementation.* **IACUC Chair or Vice Chair Printed Name:** [redacted] **IACUC Chair or Vice Chair Signature:** [redacted]	May 8, 2018 **Date:**

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

EVMS
Eastern Virginia Medical School

July 10, 2015

Gerald J. Pepe, Ph.D.
Chair, Department of Physiological Sciences
Eastern Virginia Medical School
[redacted]
Norfolk, Virginia 23507

Dear Dr. Pepe:

Your protocol entitled, ***Regulation of Fetal-placental Development in the Primate*** **(IACUC #15-009)**, was reviewed by the Institutional Animal Care and Use Committee at its July 9, 2015 meeting. **The project is now approved for one year.** Continued approval beyond this point will require submission of an annual progress report, no later than **May 10, 2016**.

PLEASE NOTE: Although the Division of Comparative Medicine (CompMed) will make every effort to provide housing, husbandry, and veterinary support for your research project, IACUC approval of this protocol does not guarantee that CompMed will be able to accommodate the work immediately. Available space and resources within the CompMed animal facility are determined by the number of ongoing projects and the animal census at any given time. Please consult with the CompMed management team to confirm the project start date.

It is the responsibility of the Principal Investigator to notify the Committee, in writing, of any deviation from the approved protocol. Please note that a change in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian prior to submitting the amendment request to the IACUC for approval.

It is also the responsibility of the Principal Investigator to provide for regular observation of the health of the research animals and to provide CompMed with the name(s) and telephone number(s) of the persons who may be contacted and/or take action in the event that the CompMed staff notes an emergency condition with the research animals.

Thank you for your continued cooperation with the Institutional Animal Care and Use Committee.

Sincerely,

[redacted]
Institutional Animal Care and Use Committee

[redacted]

Office of Research
[redacted]
NORFOLK, VA 23507
ph 757 [redacted]
fax 757 [redacted]
www.evms.edu

cc: [redacted]
Attending Veterinarian
Division of Comparative Medicine

[redacted]
Project Manager
Division of Comparative Medicine

[redacted]
Senior Associate Dean for Research
Institutional Official

EASTERN VIRGINIA MEDICAL SCHOOL
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

FOR IACUC USE ONLY:		JUN 25 2015
IACUC Number: 15-009	Review Date(s): 7/9/15	
NOTES: ORIGINAL/FINAL	Final Approval Date: 7/9/15	
	Progress Report Due: 5/10/16, 5/10/17	

Submission Instructions: Submit the original signed typed form to the IACUC Office located in [redacted], and e-mail the MSWord version of the form to the IACUC Administrator no later than 5:00 p.m. on the submission deadline date. **Forms received after the submission deadline will be held for review at the next IACUC meeting.** For assistance, please contact the IACUC Administrator at [redacted].

Initial Review Form for New Animal Care and Use Protocols

PROJECT TITLE: (If the project title is different from the grant title, please list both titles below.)
Regulation of Fetal-Placental Development in the Primate

Is this a 3-year renewal of an existing IACUC protocol?	☐ NO	☒ YES	**Related IACUC #:**	12-010

SPECIES INFORMATION: (In addition to the species, please list the strain(s), if applicable, the sex(es) and the age(s) of the animals.)
Baboon (Papio anubis/cynocephalus). Adult female (7-15 years old) and male (8-20 years old) baboons as well as offspring born to animals in the colony and studied in the pre-and post-pubertal period and as adults.

Principal Investigator (Name and Credentials):	Gerald J. Pepe, PH.D.		
Department	Physiological Sciences		
Mailing Address	[redacted]		
Office Phone #	[redacted]	Home or Cell Phone #	[redacted]
Laboratory Phone #	[redacted]	E-mail Address	[redacted]@evms.edu

Animal Emergency Contact Person:	[redacted]		
Office Phone #	[redacted]	Home or Cell Phone #	[redacted]
Laboratory Phone #		E-mail Address	[redacted]@evms.edu

Technical Coordinator:			
Office Phone #		Home or Cell Phone #	
Laboratory Phone #		E-mail Address:	

Co-Investigator #1:			
Office Phone #		Home or Cell Phone #	
Laboratory Phone #		E-mail Address	

Co-Investigator #2:			
Office Phone #		Home or Cell Phone #	
Laboratory Phone #		E-mail Address	

Co-Investigator #3:			
Office Phone #		Home or Cell Phone	
Laboratory Phone #		E-mail Address	

Co-Investigator #4:			
Office Phone #		Home or Cell Phone #	
Laboratory Phone #		E-mail Address.	

LIST ALL PROJECT SITES:					LIST THE PROJECT PERIOD:		
Bldg:	[redacted]	Room(s):	[redacted]		From:		To:
Bldg:	[redacted]	Room(s):	[redacted]		7/1/15		6/30/18

FUNDING SOURCE(S):	Please check all that apply. ☒ Federal Government ☐ State or Other Government		
	Specify the source	NIH	
	☐ Private ☐ Industry ☒ Campus/Department Funds ☐ Other		
	Specify the source		
STATUS OF FUNDING.	☒ Approved	☐ Pending	☐ Not Applicable
Is an IACUC approval verification letter needed for the funding source(s)?	☒ NO Please note that the Principal Investigator is responsible for informing the funding agency of any changes to the animal protocol. Changes to the protocol must also be approved by the IACUC before they are implemented.	☐ YES (Complete Attachment A, REQUEST FOR A LETTER OF VERIFICATION) ☐ A copy of the grant is attached. Please include a final copy of the grant to permit comparison of the animal work described in the grant with the animal work described in the Initial Review Form.	

OTHER COMMITTEE REVIEWS:

Prior to commencement of this project, approval must be acquired from the appropriate committees or offices.

Please complete the following table as it pertains to your protocol. If applicable, complete ***Attachment D, USE OF HAZARDOUS AGENTS***.

Project Involves:	Yes	No	Committee/Office	Certification Number or Approval Date	Hazard to:	
					Personnel	Animals
Radioisotopes, *in vivo*		x	EVMS Radiation Safety Committee (Complete **Attachment D**)			
Recombinant DNA, RNA, All Tissue or Cell Samples, Laboratory-induced Infection, or Cultured Pathogens	x		EVMS Institutional Biosafety Committee (IBC) (Complete **Attachment D**)	7/1/15 [redacted]	X	
Known or Suspected Chemical Hazards, Mutagens or Teratogens	x		EVMS Environmental Health & Safety Department (Complete **Attachment D**)		X	
Lasers or Penetrating Electromagnetic Radiation with Living Animals		x	EVMS Environmental Health & Safety Department			
Other (Please describe)		x				

FINANCIAL CONFLICT OF INTEREST:

If any of the activities described in this protocol represent a financial conflict of interest, I understand that I must disclose that information to the school and to the Conflict of Interest (COI) Committee as indicated in EVMS policies.

Please contact the Office of Research @ [redacted] for assistance.

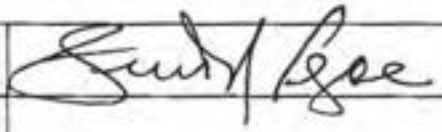
Have you, other family members, or any other person responsible for the design, conduct, or reporting of this research project:	**Yes**	**No**
(1) Received salary, other payments for services (e.g., consulting fees or honoraria), recruitment bonuses, travel expenses, or other "in kind" compensation or incentives **not directly related to the reasonable costs of conducting the research as described in the contract or agreement?**		x
(2) Received equity interests (e.g., stocks, stock options) or entitlements to the same when aggregated for you or immediate family of any amount in a publicly-traded or non-publicly traded Financially Interested Company?		x
(3) Received intellectual property rights (e.g., patents, copyrights, and/or royalty income from such rights)?		x
(4) Received any non-royalty payments or entitlements to payments in connection with the research that are **not directly related to the reasonable costs of the activity**? This includes any bonus or milestone payments to an Investigator in excess of reasonable costs incurred.		x
(5) Provided service as an officer, board member, director, or in any other fiduciary role for a Financially Interested Company, **whether or not remuneration is received for such service**?		x

PRINCIPAL INVESTIGATOR'S ASSURANCES:

I hereby certify that:

- no animal involved in this project will be subjected to discomfort, pain, or distress, unless it is unavoidable in the conduct of scientifically valuable research;
- any such discomfort, pain, or distress will be alleviated with the appropriate anesthetic, analgesic, or tranquilizing drugs, unless specific approval for not using these agents is given by the Committee;
- the project will be carried out within the provisions of the Animal Welfare Act (Public Law 99-198), the National Research Council (NRC), the Public Health Service Policy on Humane Care and Use of Laboratory Animals (PHS), the "Guide for the Care and Use of Laboratory Animals" (8th edition), the Health Research Extension Act of 1985 Public Law 99-158 (11/20/86), and United States Department of Agriculture (USDA) regulations;
- all procedural and/or personnel changes will be brought to the attention of the IACUC through the amendment process, prior to implementation, understanding that failure to request an amendment for changes in animal use may place me and the Institution in violation of federal regulations and the Animal Welfare Act;
- tails of the research to be conducted in this protocol are consistent with the details of the research as written in any grant, contract, or subcontract related to or connected with this protocol;
- all personnel using animals have completed the appropriate training requirements to assure the humane, safe, and appropriate use of animals in this context.

The signatures below signify assurance that the individuals involved will comply with the project as described herein.

Principal Investigator:		Date:	6/23/2015
Technical Coordinator:		Date:	
Co-Investigator #1:		Date:	
Co-Investigator #2:		Date:	
Co-Investigator #3:		Date:	
Co-Investigator #4:		Date:	

DEPARTMENT CHAIR'S ENDORSEMENT:
I have reviewed the research project which is the basis for this submission. I am aware of and approve of the procedures outlined in this study. I agree to take ultimate fiscal responsibility for the payment of animal care charges.

Department Chair or Designee Signature	[redacted]	Date	6-25-15
Typed or Printed Name			

VETERINARY CONSULTATION:
The IACUC requires a **mandatory** consultation with the Attending Veterinarian to provide the investigator with information that is relevant to the species and study procedures. The investigator is responsible for incorporating the appropriate information from the consultation into the Initial Review Form **before** it is submitted to the IACUC Office. **The Attending Veterinarian's signature does not constitute an approval of the protocol.** The signature merely acknowledges that a consultation with the veterinarian has occurred.

EVMS Veterinarian Signature		Date	
Typed or Printed Name	[redacted]		

IACUC APPROVAL:

IACUC Chair or Designee Signature	[redacted]	Date	July 13, 2015
Typed or Printed Name			

DEPARTMENT CHAIR'S ENDORSEMENT:
I have reviewed the research project which is the basis for this submission. I am aware of and approve of the procedures outlined in this study. I agree to take ultimate fiscal responsibility for the payment of animal care charges.

Department Chair or Designee Signature		Date	6/11/2015
Typed or Printed Name	Gerald J. Pepe Ph.D.		

VETERINARY CONSULTATION:
The IACUC requires a **mandatory** consultation with the Attending Veterinarian to provide the investigator with information that is relevant to the species and study procedures. The investigator is responsible for incorporating the appropriate information from the consultation into the Initial Review Form **before** it is submitted to the IACUC Office. **The Attending Veterinarian's signature does not constitute an approval of the protocol.** The signature merely acknowledges that a consultation with the veterinarian has occurred.

EVMS Veterinarian Signature	[redacted]	Date	6/11/15
Typed or Printed Name			

IACUC APPROVAL:

IACUC Chair or Designee Signature		Date	
Typed or Printed Name			

A. PROTOCOL OBJECTIVE:

In clear, concise, non-technical, lay language *(i.e., language understood on a sixth to eighth grade reading level and the type of writing style used in newspapers)*, summarize the background, general hypothesis, experimental plan, and relevance of the study to the advancement of scientific knowledge and/or the benefits to human and animal health. All abbreviations must be defined.

Scientific abstracts from grant applications or journal articles are not acceptable.

Goal: It is well known that the hormone estradiol, also called estrogen and produced by a women's ovary, acts on several tissues and elicits effects beneficial to women's health. Thus, cessation of estrogen after menopause increases risk for development of cardiovascular disease, diabetes, and bone loss as just a few examples. Studies, including those in our laboratories using the baboon as a model for the human, have now confirmed that estrogen also plays a critical role in pregnancy and is required for pregnancy to proceed, for the fetus/baby to grow and be delivered and ultimately develop outside the mother's womb. Uniquely in humans and nonhuman primates such as the baboon but not animals such as rodents, while the mother's ovary remains the source of estrogen during the first trimester, the placenta becomes the producer of estrogen thereafter. Moreover, placental estrogen production in primates requires participation of the fetus. Thus the fetus, mother and placenta interact and actually communicate with each other via the hormone estradiol. Unfortunately, it is impossible to perform in women invasive experiments that interrupt this maternal-fetal-placental communication to study the role of estrogen. Therefore, our understanding of the sites and means by which estrogen works and assures that the fetus grows remains incomplete. As a consequence, the incidence of premature birth, poor fetal growth, maternal diseases such as high-blood pressure/pre-eclampsia remain high. Also, unlike many years ago, women today are exposed to compounds in the environment known as endocrine disruptors several of which e.g. bisphenol A (BPA) either enhance or inhibit the actions of estradiol.

Using the baboon as a translational research model for the human, we recently showed that exposure of the mother early in pregnancy to a very small increase in estradiol significantly decreased remodeling of mom's uterine blood vessels by placental cells. This process, called uterine artery remodeling (UAR) is essential for development of normal maternal and fetal cardiovascular function in pregnancy and diseases such as pre-eclampsia in women are thought to be due to improper remodeling of the mother's spiral arteries. The present proposal outlines studies to determine the sites and mechanisms of estrogen action on the mother's uterine arteries and how fetal blood flow becomes reduced/compromised when the maternal vessels are poorly remodeled. If blood is not adequately supplied to the placenta, the fetus does not get maternal nutrients including oxygen and foods like glucose and thus does not grow normally and also becomes oxygen-deprived and has poor vascular function. We also propose studies to determine whether the negative impact of defective UAR on fetal blood flow is still apparent when the fetus is born and develops as an adult. By knowing how and the sites/factors regulated by estrogen, we can design studies in women to begin to determine who might be at risk and design methods/approaches to reduce the impact of the disease.

Our laboratories also showed that inhibition of the increase in placental estradiol production in the second half of pregnancy altered development of key organs in the fetus including the adrenal gland. In addition, babies born to mothers in which placental estradiol was suppressed exhibited a reduced response to insulin, a condition known as insulin resistance. These findings indicate that insulin resistance which leads to diabetes or uncontrolled high blood sugar may have its origins in the womb and that estrogen acts on fetal tissues to prepare them to respond to insulin when the individual is an adult. We call this effect of estrogen, programming. The sites and mechanisms by which estrogen is working to program the fetus is a goal of the second series of experiments outlined in this protocol.

To accomplish our goals and study the role of estrogen in pregnancy, pregnant baboons will be treated with estradiol or a specific inhibitor of estrogen synthesis alone or in combination with estrogen. Treated/untreated animals will be delivered by cesarean section at early, mid or late gestation and the placenta and fetal tissues collected and studied for aspects of biochemical/physiologic maturation. In other experiments, treated/untreated animals will be delivered near term and neonates reared to adulthood. Development of vascular function (e.g. ability to control blood pressure), blood vessel flow and glucose (sugar) regulation as indexes and/or predictions of development of diabetes will also be determined. These studies serve as a model for the human and are designed to provide new information which will enhance our understanding of the causes of pregnancy complications in women (e.g. preeclampsia; fetal growth retardation and prematurity *per se*) and the role of hormones *in utero* on programming fetal organ systems critical for development of appropriate vascular (e.g. blood pressure), and metabolic (e.g. glucose-diabetes) function in adulthood.

B. SEARCH FOR ALTERNATIVES:

In an effort to minimize pain and distress, the Animal Welfare Act (AWA) regulations require Principal Investigators (PIs) to consider alternatives to procedures that may cause more than momentary or slight pain or distress to animals. The AWA also requires PIs to provide a written narrative of the methods used and sources consulted to determine the availability of alternatives, including replacement, reduction, and refinement of animal use. These alternatives should be consistent with the goals of the proposed research. *Potential alternatives that do not allow the attainment of the goals of the proposed research are not, by definition, alternatives.* The "3 Rs" are defined below:

REPLACEMENT: *An alternative that will be equally informative.* Replacements include, but are not limited to, *in vitro* models, in silico methods, invertebrate models, and vertebrate models.

REDUCTION: *Reducing the number of animals to the minimum required to obtain scientifically valid data and demonstrating that the proposed research does not unnecessarily duplicate previous work.* Reduction includes statistical methods to reduce animal numbers, and it addresses whether or not animals can be reused for other purposes.

REFINEMENT: *A procedure that lessens or eliminates pain or distress, thereby enhancing animal well-being.* Housing, environmental enrichment, animal identification, anesthesia, analgesia, and euthanasia procedures can be refined, in addition to activities normally thought of as procedures, such as surgeries, tissue or fluid collection, etc.

The fundamental goal of the AWA and USDA Policy #12 is to minimize pain and distress to animals; consequently, the regulations state that any proposed animal activity or significant changes to an ongoing animal activity must include the following: (1) a rationale for involving animals, and the appropriateness of the species and the number of animals to be used; (2) a description of the procedures or methods designed to assure that discomfort and pain to animals will be limited to that which is unavoidable in the conduct of scientifically valuable research, and that analgesic, anesthetic, and tranquilizing drugs will be used where indicated and appropriate to minimize discomfort and pain to animals; (3) a written narrative description of the methods and sources used to consider alternatives to procedures that may cause more than momentary or slight pain or distress to the animals, and; (4) the written assurance that the activities do not unnecessarily duplicate previous experiments.

DATABASE SEARCHES

A database search is considered to be the most effective and efficient method for demonstrating compliance with the federal regulations for consideration of alternatives to painful and distressful procedures, although other sources, such as conferences, colloquia, subject expert consultation, etc., may provide relevant and up-to-date information regarding alternatives, in lieu of or in addition to a database search. **Institutional policy requires investigators to specify at least two (2) databases or other acceptable sources** that were used to determine that alternatives to animals have been considered, that the minimal number of animals have been requested, that the proposed research is not duplicative of previous work, and that alternatives to procedures that may cause more than momentary or slight pain or distress to the animals have been considered. **For all database searches, the following information must be provided: (1) the name of the database; (2) the date the search was performed; (3) the time period covered by the search, and; (4) the key words and/or the search strategy used.**

Please be sure to list all key words and key word combinations used and the number of citations found for each key word or combination [*e.g., amiloride mouse kidney (455 citations), mouse hemizona assay (453 citations)*]. **PLEASE NOTE: The search must include the key word "pain" and any relevant combination thereof.** Be sure to search for all applicable terms, including the search for alternatives [*e.g., mouse heart computer model (55 citations)*]. Use the possible time range possible to include both modern and classical references. **A member of the EVMS Brickell Medical Sciences Library Services staff is available to assist with the searches.**

EXPERT CONSULTATIONS

An appropriate, well documented consultation with an expert in the field of the proposed research can replace a second database search. In order to demonstrate to the IACUC the expert's knowledge of the availability of alternatives in the specific field of study, documentation of the consultation **must** include the following: (1) the consultant's name and qualifications; and (2) the date and content of the consultation **as** it relates to replacement, reduction, and/or refinement.

DESCRIPTION AND JUSTIFICATION

Regardless of the sources used to search for alternatives, the written narrative should include adequate information for the IACUC to assess that a reasonable and good faith effort was made to determine the availability of alternatives to animals or procedures. If the database search or another acceptable source identifies an alternative that could be used to accomplish the goals of the proposed research; however, the investigator chooses not to use that alternative, the investigator must provide a written narrative justifying why the alternative was not used.

1. **Database and Literature Searches:**

	Yes (X)	Date Search Conducted	Key Words/Search Strategy	Time Period Covered by the Search
Databases/Computer Systems				
AGRICOLA Database (National Agriculture Libr.)				
MEDLINE Database Searcher – [redacted]	X	6/5/15	Estrogens/estrogen receptor modulators, etc (25729); embryonic/fetal growth/development (43037); gonad, testis, adrenal glands, adipose tissue, skeletal muscle, placenta, pancreas, etc (35217); menstrual cycle, pre-eclampsia, insulin resistance, fertility, angiogenesis, etc (201061); cesarean section-complications (2480); papio/baboons (803); animal welfare, animal models, pain/stress/distress/suffering, refine/replace/reduce, humane endpoint, etc. (1711841). **Citations = 9, 3**	2012-2015 3 yr update
CAB Abstracts Database				
TOXLINE				
BIOSIS Database				
Other: Web of Science (Science Citation Index) Searcher – [redacted]	X	6/5/15	Estrogens/estrogen receptor modulators, etc (28081); embryonic/fetal growth/ development (54774); gonad, testis, adrenal glands, adipose tissue, skeletal muscle, placenta, pancreas, etc (45147); menstrual cycle, pre-eclampsia, insulin resistance, fertility, angiogenesis, etc (97118); cesarean section-adverse effects (5524); papio/baboons (1415); animal welfare, animal models, pain/stress/distress/suffering, refine/replace/reduce, humane endpoint, etc. (2236369). **Citations = 8, 0**	2012-2015 3 yr update
Literature and Reference Sources				
AAALAS				
Quick Biblio. Series (AGRICOLA)				
Laboratory Animal Welfare Biblio. (NLM)				

Animal Welfare Information Ctr				

2. List any consultations with investigators in the field. The consultation(s) should be related to replacements, reductions, and/or refinements and not simply to the science behind the research. (This information is not required if two database searches were performed and documented above.)

N/A

3. Provide a brief narrative regarding search methods used, but not listed above.

The research outlined in this protocol has consistently developed and been supported in large part by NIH R01 HD 13294 (1980-2013), U54 HD 36207 (1997-2010) and more recently by NIH DK 093590 (2013-2017). Although HD 13294 is no longer funded by NIH, a significant aspect of work/studies developed under the auspices of this grant are still in progress and funded by departmental sources and data being published and used as supportive rationale/preliminary data for new grant submissions to the NIH. All previous grant submissions have consistently been viewed as exhibiting outstanding clinical/translational relevance to the human. Since 1981, the research program using the pregnant baboon model to study placental-fetal development has resulted in publication of over 150 manuscripts in peer-reviewed journals with high impact factors (e.g. Endocrinology) as well as seminal review chapters in "Endocrine Reviews". In addition, a search of the literature was performed in consultation with [redacted], librarian at EVMS. The databases searched included: Medline and Web Of Science (Science Citation Index) and employed key words most notably baboons, humane endpoint, refine/reduce/replace, placenta, pain/distress/ stress/suffering, preeclampsia, insulin resistance, cesarean section complications, animal models, and animal welfare. The Medline search also employed several other key words (e.g. estrogens/estrogen receptor modulators antagonists, estrogen receptors). The initial search history (2012-2015; 3 year update) identified (depending on key word) anywhere from 803 to over 2,236,369 results; a refined search of these hits indicated that none outlined an alternative procedure for the studies we have outlined in our protocol. Moreover, of the several manuscripts cited/abstracts printed as relevant to the search questions, several were publications from my laboratory. Although studies using rodents were identified, the rodent (including rat, mouse, guinea pig) is not an acceptable model for studies of human placental-fetal development. Thus, these animals do not have a fetal-placental unit, do not exhibit fetal organ system maturation as occurs *in utero* in the human (e.g. adrenal gland, ovary or testis) and exhibit unique patterns of postnatal development that impact organ system development also not typically noted in the human. Most importantly, type of placenta and thus transfer of maternal substrates across the placenta to the fetus, as well as the fetal hormonal milieu of rodents and even large farm animals (e.g. sheep) are significantly different from that in the human. In contrast, and as substantiated by the literature search, the baboon is a well-established model for studies of human pregnancy.

C. NARRATIVE: *The narrative must address the following:*

4. Provide the rationale/justification for animal use. Discuss the alternatives (*e.g., cell lines, computer simulations, or artificial bodies*) that were considered.

There is no way to perform these studies in humans and the potential impact on the development of new treatment regimens for maternal/fetal medicine is profound. For example, based primarily on epidemiologic evidence as well as *in vitro* studies it has been proposed that defective UAR underlies ischemic placental disease and which is thought to be the cause of intrauterine growth restriction (IUGR), early-onset pre-eclampsia, and preterm birth. Unfortunately, while invasion of the uterine spiral arteries may indeed be the cause of these disease the combined incidence of which affect up to 15% -20% of all pregnancies, no one has been able to demonstrate cause:effect. Recently (March, 2015) NIH put out an RFA requesting proposals to not only come to understand regulation of placental development including UAR, but to develop new imaging technologies that can measure UAR and when it is defective whether that can be detected early in the pregnancy before onset of disease which typically occur later in the pregnancy. Our animal model is ideal for that and we have recently (June 1, 2015) responded and submitted a proposal in response to the RFA. Our studies show that too much estrogen early in pregnancy may be a causative factor and women in IVF (ART) programs almost always have extremely high levels of estradiol and progesterone in early stages of their pregnancies and the risk for having an IUGR baby or mother developing preeclampsia are much higher in IVF pregnancies than normal. Other animals, such as rodents, cannot be used for such studies as they differ (e.g. placentation) considerably from the human. The baboon, like humans has a fetoplacental unit and a similar hormonal profile, placental development and metabolic machinery and fetal adrenal and ovarian anatomy, biochemistry and developmental pattern. Moreover, examination of isolated human tissue or computer simulations does not permit elucidation of the interaction between mother, placenta, and fetus in pregnancy maintenance/fetal development/parturition. Moreover, such studies do not permit testing that what happens *in utero* actually impacts physiological outcome in adulthood. As emphasized in several journals, fetal growth restriction, prematurity, pre-eclampsia and infertility as well as diabetes and hypertension/ cardiovascular disease continue to be major health problems in the United States with annual direct costs associated with fetal immaturity alone exceeding that caused by AIDS. In humans, a poorly developed or inadequately functioning placenta results in intrauterine growth

retardation/reduced neonatal birth weight and epidemiologic studies have shown that adults with low birth weight are predisposed to hypertension and reproductive dysfunction. While these clinical studies cannot provide cause:effect information, they may become more meaningful when interpreted in light of results from our *in vivo* studies in the baboon. Clearly, the experimental baboon model and the multidisciplinary collaborative approach developed by the investigators permit a necessary evaluation of the interactions essential to fetal-placental development. Thus, the results derived from the completion of this study will provide important new insight into the communication that occurs between the fetus and placenta and ultimately improve our knowledge of the regulation of pregnancy maintenance and development of neonatal self-sufficiency and reproductive function in the human. Finally, the search of the databases outlined in Section B question 3, did not identify any alternative methods/procedures for conduct of studies to examine role of pregnancy on placental-fetal development and impact of the hormonal milieu *in utero* on physiologic/reproductive function in adulthood. Rather, the search identified manuscripts that demonstrated the value and acceptability of the baboon as a model for studies of human pregnancy

5. **Discuss the appropriateness of the species (and the animal strain, if applicable) chosen to meet the objective(s) of the study.**

In the present study, we propose to continue our use of the pregnant baboon as a model to study the developmental regulation of maternal, uteroplacental, and fetal adrenocortical, skeletal muscle and hepatic maturation and function in human pregnancy as well as impact of the intrauterine hormonal milieu on neonatal growth and physiologic (e.g. glucose tolerance; vascular function) function in adulthood. Because the maternal, fetal, and placental units are functionally interrelated during human and nonhuman primate pregnancy, e.g. estrogen biosynthesis, they cannot be evaluated separately. Therefore, *in vitro* approaches utilizing isolated tissues do not on their own permit an assessment of the maternal-fetal-placental endocrine system. As in humans, the baboon possesses a hemochorial and monodiscoid placenta and a functional fetoplacental unit, in which the fetal adrenal gland provides the major portion of C_{19}-steroid precursors required for placental estrogen formation. Because non primate laboratory animals, e.g. the laboratory rat, do not exhibit hemochorial placentation and do not possess a fetoplacental unit for the biosynthesis of hormones such as estrogen and maturation of fetal organ systems including the adrenal occurs post-natally (i.e. extra-uterine), their applicability to the human is limited.

The qualitative and quantitative hormonal profiles exhibited in pregnant baboons are also similar in many important respects to those in pregnant women. For example, the progesterone production rate and serum progesterone concentrations are elevated during pregnancy in baboons as in women. This contrasts with other nonhuman primates, e.g. rhesus monkeys, in which serum progesterone concentrations and production rates are similar in the pregnant and nonpregnant states. An elevation in the quantities of progesterone in the peripheral circulation is essential to enable their manipulation and thus study of the regulatory factors involved. Similarities in the metabolism of progesterone during baboon and human pregnancy further support the use of the baboon for the study of steroid hormone production. Thus, the major metabolite of progesterone in women and baboons is pregnanediol, while in rhesus monkeys it is androstenedione. The concentrations and patterns of estradiol and estrone in the maternal circulation of pregnant baboons are similar to those in pregnant women, while the concentration of estradiol in rhesus monkeys at term is an order of magnitude less than in women. Corticosteroid production and metabolism also are similar in female baboons and humans. Indeed, the rate of cortisol production and excretion, type and degree of conjugation and formation of tetrahydrocortisol and tetrahydrocortisone as major metabolites are very similar in baboons and women. This contrasts with other nonhuman primates including most new-world primates (owl, squirrel and marmoset monkeys) in which serum cortisol levels and production rates are excessively high and comparable to those in humans with Cushing Syndrome.

Therefore, the baboon provides an excellent, scientifically valid model for the study of the endocrinology of human pregnancy. Finally, the >30 years of baseline data which this laboratory has obtained in pregnant baboons forms a critically important basis for the continued use of this animal model and further points to the value and peer-reviewed acceptance of the baboon for the study of the endocrinology of human pregnancy. Moreover, the search of the databases outlined in Section B question 3, did not identify any alternative methods/procedures for conduct of studies to examine role of pregnancy on placental-fetal development and impact of the hormonal milieu *in utero* on physiologic/reproductive function in adulthood. Rather, the search identified manuscripts that demonstrated the value and acceptability of the baboon as a model for studies of human pregnancy.

The numbers used are the minimum to permit collection of statistically valid and scientifically meaningful data. Sample size for comparison of means by treatment was determined by estimating the variance as from previous studies (1972-10) in my laboratory and assuming the populations are normally distributed obtained as outlined in Daniel (Biostatistics: A Foundation Analysis in the Health Sciences, 4th Ed., 1987).

6. Describe the steps taken to reduce the number of animals required for the study (*e.g., replacement with in vitro procedures, refinement of experimental design, refinement of procedural techniques*).

As indicated above, there is no way to perform these studies in humans and the potential impact on the development of new treatment regimens for maternal/fetal medicine is profound. Other animals, such as rodents, cannot be used as they differ (e.g. placentation) considerably from the human. The baboon, like humans has a fetoplacental unit and a similar hormonal profile, placental metabolic machinery and fetal adrenal anatomy/biochemistry. Moreover, examination of isolated human tissue or computer simulations does not permit elucidation of the interaction between mother, placenta, and fetus in pregnancy maintenance/fetal development/ parturition. We have refined our experimental designs such that we use the same animal preparation to examine the role of estrogen on placental as well as fetal development and maternal well-being. In other words, we do a single primary experimental manipulation (e.g. injection of estrogen) and monitor the mother throughout the pregnancy (e.g. ultrasound; peripheral blood sampling for hormone and blood chemistries) and examine several aspects of placental (e.g. endovascular invasion; placental microvilli) and fetal organ system (e.g. skeletal muscle; gonad; adrenal; liver; pituitary) development and function. Finally, the search of the databases outlined in Section B question 3, did not identify any alternative methods/procedures for conduct of studies to examine role of pregnancy on placental-fetal development and impact of the hormonal milieu *in utero* on physiologic/reproductive function in adulthood. Rather, the search identified manuscripts that demonstrated the value and acceptability of the baboon as a model for studies of human pregnancy.

7. Will the animals be subjected to procedures that may cause more than momentary or slight pain or distress? [**NOTE:** *These procedures include environmental, nutritional, or behavioral modifications that increase stress, as well as chronic food or water deprivation.*]

X Yes (A database search is required. Complete Question 8) ___ NO (Skip to **Question 9**)

8. If alternative procedures were identified, describe the procedures below and explain why they are not scientifically appropriate for this research project.

No. The search of the databases outlined in Section B question 3, did not identify any alternative methods/ procedures for conduct of studies to examine role of pregnancy on placental-fetal development and impact of the hormonal milieu *in utero* on physiologic/reproductive function in adulthood. Rather, the search identified manuscripts that demonstrated the value and acceptability of the baboon as a model for studies of human pregnancy.

9. Is the proposed study duplicative of research previously undertaken by the investigator or other scientists? **If yes, describe the duplicative nature of this project and provide scientific justification for completing this study.**

No. Thus, the search of the databases outlined in Section B question 3, did not identify any alternative methods/ procedures for conduct of studies to examine role of pregnancy on placental-fetal development and impact of the hormonal milieu *in utero* on physiologic/reproductive function in adulthood. Rather, the search identified manuscripts that demonstrated the value and acceptability of the baboon as a model for studies of human pregnancy.

10. Federal regulations require a written rationale/justification for the number of animals requested to complete this study. Describe the statistical test (*e.g., power analysis and/or other rationale, such as tissue collection needs and breeding efficiency*) used to determine the number of animals required to complete the proposed study, and provide the results of the test.

NOTE: The IACUC may require a consultation with a statistician.

Throughout the course of conduct of our studies we have consulted a statistician at EVMS (or at the University of Maryland). For example, for analysis of the number of samples required to ascertain whether there are statistical differences ($P<0.05$) between populations in tissue morphology and/or expression of mRNA and/or protein biochemical measures (e.g. estrogen receptor mRNA/unit housekeeping gene) using analysis of variance with post hoc comparison of means by the Neumann-Keuls statistic and appropriate transformation of variables to satisfy ANOVA assumptions, there will be > 80% power to identify differences between the 3 or 4 treatment groups with n=8/group ($\emptyset = 2.0$). For analysis of histology and levels of factors in tissue samples from conduct of Studies I and II, statistical differences ($P<0.05$) between populations in tissue morphology (i.e. number of spiral arteries remodeled/total number of spiral arteries; placental microvillus number/height) and biochemical development (i.e. GLUT-4 protein and mRNA levels) will be determined by analysis of variance with *post hoc* comparison of the means by the Newman-Keuls statistic and appropriate transformation of variables to satisfy ANOVA assumptions.

between the 3 or 4 treatment groups. For the acute glucose tolerance tests stimulation and *in vitro* studies of adrenal function comparison of data at different time points will consist of a repeated measures mixed-model ANOVA with treatment as fixed effect and subject as random effect.

D. USDA PAIN CODES:

11. For each of the appropriate pain code descriptions, list the species (and the animal strain, if applicable) and the number of animals to be used each year. **Please provide the 3-year total for each pain code level.**

Level B				
Breeding or holding colony protocols where animals do not undergo any manipulation. **Unused pups generated during breeding should be included in Level B.**				
Species	**Year 1**	**Year 2**	**Year 3**	**Total**
Baboon (*Papio anubis/cynocephalus*) adult male breeders and spontaneously aborted/still birth fetus	6	6	6	18

Level C				
Teaching, research, experiments, or tests conducted on animals involving no or momentary/slight pain or distress (*e.g., euthanizing animals for tissues; injections; observation under normal conditions; positive reward projects; use of Acepromazine for vasodilatation in rabbits*) and **for which no pain-relieving drugs are used.**				
Species	**Year 1**	**Year 2**	**Year 3**	**Total**
Baboon (*Papio anubis/cynocephalus*)	21	21	22	64

Level D				
Teaching, research, experiments, surgery, or tests conducted on animals involving a degree of pain or distress (*e.g., non-survival surgery; survival surgery; antibody production; subcutaneous implants; induced infections*) and **for which appropriate anesthetic, analgesic, or tranquilizing drugs are used to relieve pain and distress**.				
Species	**Year 1**	**Year 2**	**Year 3**	**Total**
Baboon (*Papio anubis/cynocephalus*) Female adults and juveniles	28 adult females and 13 EVMS born	28 adult females and 13 EVMS born	28 adult females and 14 EVMS born	84 adult females and 40 EVMS born

Level E				
Teaching, research, experiments, surgery or tests conducted on animals involving a degree of pain or distress and **for which the appropriate anesthetic, analgesic or tranquilizing drugs are NOT used because their use will adversely affect the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests**. **(SCIENTIFIC JUSTIFICATION IS REQUIRED)**				
Species	**Year 1**	**Year 2**	**Year 3**	**Total**
None				
Below, please provide scientific justification for use of Level E animals:				

E. STUDY PROCEDURES:

12 Please indicate all procedures to be performed in this study. **(Attach all required forms)**

X	Non-Survival Surgery (Complete **Attachment E, ANIMAL SURGERY**)
X	Single Major or Minor Survival Surgery (Complete **Attachment E, ANIMAL SURGERY**)
X	Multiple Major Survival Surgery (Complete **Attachment E, ANIMAL SURGERY**)
	Prolonged Restraint (Complete **Attachment C, PROLONGED PHYSICAL RESTRAINT OR STRESS**)
X	Collection of Tissues, Cells, or Organs
	Adverse Conditioning
	Special Diet
	Food/Water Deprivation (Complete **Attachment C, PROLONGED PHYSICAL RESTRAINT OR STRESS**)
X	Use of Biohazards or Chemical Agents (Complete **Attachment D, USE OF HAZARDOUS AGENTS**)
	Burns or Trauma
	Antibody Production (Complete **Attachment F, ANTIBODY PRODUCTION**)
	Use of X-Rays or Other Radiation
	Tumor Transplantation/Induction
	Toxicity Testing (LD-50) (Complete **Attachment G, DEATH AS AN ENDPOINT**)
x	Use of Non-pharmaceutical-grade Chemicals or Other Substances (Complete **Question 17a**)
x	Use of DEA Controlled Substances (Complete **Question 15**)
	Photographs and/or Videos (See the CompMed ***Camera/Cell Phone Use Policy***)
X	Teaching or Training Protocol (Complete **Question 12a**)

12a. If this is a teaching or training protocol, please check all that apply

	Undergraduate or graduate students
	Continuing education students (M.D.)
X	Only dead animals or tissues obtained through euthanasia by the PI will be used.
	Demonstration (PI only performing procedures)
	Student involvement (Students performing/assisting with procedures)
	Use within a Biomedical Sciences Course (*ID #/Name:* _____)
	Other (Please explain)

F. RESEARCH DESIGN:

13. The IACUC reviewers are scientifically knowledgeable, however, they may not be experts in your specific field of study. Please provide a brief (i.e., one or two paragraphs) overview of the project design and how each experimental goal relates to the project design. The description should provide a sequential overview of all procedures and should account for each animal subject by experimental group. The overview should be followed by a chronological description of all experimental procedures related to the care and use of the animals. **The use of tables and flow charts to organize the procedures, numbers of animals, and schedules is recommended.** Do not paste in method sections from grant applications or journal articles. Do not include methods pertaining to *in vitro* work, unless it applies to the care and use of animals. For each animal or experimental group, provide information on the duration of each procedure (*i.e., fluid or tissue collections, methods, sites, volumes/weights, frequencies, etc.*) and the total time from initial contact to completion. **Although procedures involving drug manipulations and surgery are detailed in other sections of this form, their application in the research design should be stated here. Any procedures not covered in later sections of this form must be completely detailed in this section.**

By reading only this section of the Initial Review Form, the IACUC should be able to clearly determine each experiment being performed on each individual animal.

General overview of the project:

The overall goal of the project is to elucidate the role of estrogen in primate pregnancy on development of the fetus/placenta and impact on physiologic processes in the offspring. Over the past 30 years this laboratory using the baboon as a model for human pregnancy, has shown that estrogen is a key hormone important for placental and fetal development. Moreover, our studies have shown that critical organ systems as well as metabolic processes in the fetus appear to be programmed by estrogen, consistent with the new prevailing theory that in addition to our genetic makeup, who/what we are physiologically as adults is established *in utero* by epigenetic mechanisms (e.g. programming). Thus, interference with this intrauterine programming either by premature birth, poor fetal growth, exposure to environmental factors that interfere with and or enhance estrogen action increase the risk for development in adulthood of diseases such as hypertension and diabetes. Thus, it is critical to understand what estrogen is doing. However, in examining the role of estrogen it is important to recognize that the source and levels of this hormone change during pregnancy. During the first trimester (days 1-60 in the baboon; term = 184 days), the maternal ovary is the source of estrogen and maternal (as well as fetal) estradiol levels are typically relatively low and more like that during the follicular phase of the mother's menstrual cycle (i.e. <300 pg/ml). At the end of the first trimester, the placenta takes over and becomes the source of estradiol. As a result, the maternal (and fetal) levels of estradiol increase daily throughout the second half of gestation and by term exceed 5,000 pg/ml (fetal estrogen levels are about 20% of those in the mother). Based on our studies and proposed new experiments, too much estrogen early in gestation (e.g. as can occur in '*in vitro*" pregnancies; exposure of mother to estrogen like-molecules in the environment) or interference with the availability/action of estrogen during the second half of gestation (exposure to environmental inhibitors of the estrogen receptor; premature delivery) are equally harmful to placental/fetal development and physiologic function in adulthood.

STUDY I: ROLE OF ESTROGEN IN EARLY GESTATION

During the period of relatively low estrogen, numerous events occur that are essential for establishment of a successful pregnancy. Notably, the placenta and fetus must develop blood vessels, gain accessibility to nutrients in mother's blood and coordinate/regulate blood flow. To accomplish these things, cells in the newly developing placenta, specifically the extra villous trophoblast (EVT) migrate and attach to the uterine spiral arteries that supply mother's blood to the uterus and products of conception. These placental cells erode about 80%-90% of the smooth muscle that comprise the blood vessels. Moreover, about 50%-70% of the vessels are "invaded" by the placental cells a process termed remodeling and which renders these vessels unable to respond to vasoactive agents and thus do not contract (e.g. when mom gets anxious/stressed). Thus, the invaded vessels are transformed from low-capacity-high resistance to high capacity-low resistance vessels and blood just "dumps" into the placental intervillous space and serves as a reservoir of nutrients (e.g. oxygen; glucose) for the fetus. The vessels offer no resistance to flow and as a result do not significantly influence mother's blood pressure. This critical process is essentially over by the middle of the second trimester. But what regulates this and why does it end at this time? This is a critical question because we now know that in women who develop the pregnancy complications, preeclampsia and intrauterine growth restriction, there is "shallow placental invasion", i.e., the mother's uterine arteries are not adequately invaded by the placental cells. Preeclampsia is life threatening and often complicated by increased maternal blood pressure and reductions/complications in placental and fetal blood flow that severely restrict fetal growth. During the previous project period, we showed that by simply injecting the baboon mother in the first trimester with estradiol and increasing estrogen levels to those normally seen at the beginning of the second trimester, we inhibited placental production of vascular endothelial growth factor (VEGF), blocked placental invasion of the uterine arteries, and disrupted placental/fetal blood flow and response of the fetal-placental vessels to the vasoactive agent serotonin. In the current project period we propose experiments to determine the

mechanism by which estrogen elicits these effects and ascertain impact on placental and fetal development and whether changes in fetal blood flow persist into adulthood and thus were programmed *in utero*. Briefly, female baboons within our primate colony are mated with male baboons of proven fertility, pregnancy confirmed by ultrasound or absence of sex skin swelling and menses.

Experimental Treatment Groups:

Group 1: Untreated; day 60, placenta/fetus delivered, euthanized and studied (n = 8)
Group 2: Maternal estradiol daily on days 25-59; day 60, placenta/fetus delivered, euthanized and studied (n = 8)
Group 3: Untreated; maternal studies - days 80, 100, 150, 160; day 175 placenta/fetus delivered, euthanized and studied (n = 8)
Group 4: Maternal estradiol daily days 25-59; maternal studies- days 80, 100, 150, 160; day 175, placenta/fetus delivered, euthanized and studied (n = 8)
Group 5: Untreated; maternal studies-days 80,100,150,160; deliver spontaneously; offspring reared and metabolic and vascular function studied pre and postpuberty (n = 8)
Group 6: Maternal estradiol-daily days 25-59; maternal studies-days 80,100,150,160; deliver spontaneously; offspring reared and metabolic and vascular function studied pre and postpuberty (n=8)

Study I Maternal Experiments

N=	Treatment Group	Blood Sampling	*IVGTT(under ketofol, O_2 via intubation)	FMD (under ketofol, O_2 via intubation)	Serotonin infusion (fetal flow Doppler under ketofol, O_2 via intubation)	Delivery Status (under isoflurane / O_2 intubation)
16	With or without estradiol benzoate (25µg/kg / 1ml sesame oil, sc	2-4 day intervals under ketamine sedation				C-section ~60d fetus euthanized
16	With or without estradiol benzoate (25µg/kg / 1ml sesame oil, sc	2-4 day intervals under ketamine sedation	early (~80-120d) and late (~140-160d) gestation	early (~80-120d) and late (~140-160d) gestation	once late gestation prior to c-section (~160-170d)	C-section ~165-175d fetus euthanized
16	With or without estradiol benzoate (25µg/kg / 1ml sesame oil, sc	2-4 day intervals under ketamine sedation	early (~80-120d) and late (~140-160 d) gestation	early (~80-120d) and late (~140-160 d) gestation		Spontaneous delivery neonate survives**

* ivGTT = intravenous glucose tolerance test; FMD = test flow mediated dilation of brachial artery]
** Studies in offspring described on p. 18-19

Total number of pregnancies for Study I: N = 48 (8/group x 6 groups)
Total number of neonates/offspring delivered/reared for Study I: N = 16 (8/group x 2 groups)

STUDY II: ROLE OF INCREASING LEVELS OF ESTROGEN DURING THE SECOND HALF OF GESTATION

During the second half of pregnancy in humans and baboons, there is a tremendous increase in estrogen production by the placenta accompanied by significant growth, differentiation and maturation of the fetus and the placenta. Our laboratories have shown that this increase in estradiol is essential for structural and functional maturation of the placenta (e.g. microvilli; enzymes controlling cortisol metabolism) and organ systems in the fetus including the fetal ovary and adrenal gland. Moreover, estrogen also appears to program tissues/organ systems of the fetus that impact insulin sensitivity. Interest in estradiol in pregnancy is heightened as mentioned above by studies showing that factors which interfere with the availability or action of estradiol increase risk for development of disease including diabetes in adulthood. Our overall goal is to ascertain the mechanisms by which estrogen regulates development of the primate fetal adrenal gland and development of insulin sensitivity in adulthood. To test our hypotheses, we treat pregnant baboons with an aromatase inhibitor (letrozole) without/with estrogen to reduce/restore estrogen production during the second half of gestation and remove fetal tissues, e.g. adrenal glands, skeletal muscle, fat, liver, lung, heart and the pancreas to study expression of proteins that are essential for insulin action. *In vitro* studies are also performed to determine the mechanism of estrogen action including incubation of fetal adrenal cells with ACTH in presence/ absence of estradiol or the estradiol receptor agonists and/or inhibitors of downstream signaling molecules. We also examine maternal glucose homeostasis (e.g. glucose-tolerance tests; fasting insulin) and other hormones/factors (e.g. androgens, cortisol, cytokines) to confirm that alterations in the fetal adrenal, ovary or fetal tissue glucose tolerance/ insulin sensitivity are not the result of modification of maternal mechanisms. Finally, neonates born to mothers treated *in utero* with nothing, aromatase inhibitor ± estrogen are raised to adulthood (puberty at 36-48 months of age) and adrenal function as well as glucose tolerance/insulin action and microvascular blood flow by Doppler contrast enhanced ultrasound studied prior to and after onset of puberty to determine impact of estrogen programming of fetal adrenal and fetal metabolic systems on metabolic and adrenocortical function in adulthood.

Briefly, female baboons within our primate colony are mated with male baboons of proven fertility, pregnancy confirmed by ultrasound and/or fetal sex determined by chromosomal analysis of fetal cells isolated from amniotic fluid obtained at day ~80-100 of gestation (term = day 184). Procedure is performed during initial IVGTT to reduce number of sedations.

Experimental Treatment Groups:

Group 1: Untreated; maternal ivGTT and FMD* day 80 and 90; fetus delivered and studied day 100 (n = 8)*

Group 2: Untreated; maternal ivGTT (day ~80 and ~150) and FMD (day ~100 and ~160); fetus delivered/studied day 165-170 (n = 8)
Group 3: Maternal letrozole daily days 100-170; maternal ivGTT (day ~80 and ~150) and FMD (day ~100 and ~160); fetus delivered/studied day 165-170 (n = 8)
Group 4: Maternal letrozole + estradiol daily days 100-170; maternal ivGTT (day ~80 and ~150) and FMD (day ~100 and ~160); fetus delivered/studied day 165-170 (n = 8)
Group 5: Untreated; maternal ivGTT (day ~80 and ~150) and FMD (day ~100 and ~160); mothers deliver spontaneously and offspring metabolic, vascular and adrenal function determined before and after puberty (n = 8)
Group 6: Maternal letrozole daily days 100-170; maternal ivGTT (day ~80 and ~150) and FMD (day ~100 and ~160); mothers delivered via c-section and offspring metabolic, vascular and adrenal function determined before and after puberty (n = 8)
Group 7: Maternal letrozole + estradiol daily days 100-170; maternal ivGTT (day ~80 and ~150) and FMD (day ~100 and ~160); mothers deliver spontaneously and offspring metabolic, vascular and adrenal function determined before and after puberty (n = 8)

Total number of pregnancies for Study II: N = 56 (8/group x 7 groups)
Total number of neonates delivered/reared for Study II: N = 24 (8/group x 3 groups)

Study II Maternal Experiments

N=	Treatment Group	Blood Sampling	*IVGTT(under ketofol, O_2 via intubation)	FMD(under ketofol, O2 via intubation)	Serotonin infusion (fetal flow Doppler under ketofol, O_2 via intubation)	Delivery Status (under isoflurane / O_2 intubation)
8	No treatment	2-4 day intervals under ketamine sedation	early (~80-120d) and late (~140-160d) gestation	early (~80-120d) and late (~140-160d) gestation		C-section ~100d fetus euthanized
24	With or without letrozole / letrozole +estradiol (~100-170d) both 115µg/kg	2-4 day intervals under ketamine sedation	early (~80-120d) and late (~140-160d) gestation	early (~80-120d) and late (~140-160d) gestation		C-section ~165-175d fetus euthanized
16	With or without letrozole +estradiol (~100-170d) both 115µg/kg	2-4 day intervals under ketamine sedation	early (~80-120d) and late (~140-160d) gestation	early (~80-120d) and late (~140-160d) gestation		Spontaneous delivery neonate survives
8	With letrozole (~100-170d) 115µg/kg	2-4 day intervals under ketamine sedation	early (~80-120d) and late (~140-160d) gestation	early (~80-120d) and late (~140-160d) gestation		C-section ~165-175d neonate survives **

* ivGTT = intravenous glucose tolerance test; FMD = test flow mediated dilation of brachial artery]
** Studies in offspring described on p. 18-19

Overall total number of pregnancies for Studies I and II: N = 104
Total number of neonates/offspring delivered/reared for Studies I and II: N = 40

This research program continues to function as a collaborative effort with colleagues at the University of Maryland as has occurred over the past 30 years. Thus, approximately 50% of the studies/animal treatments will be performed at the University of Maryland and 50% at EVMS and tissue samples shipped between Institutions. Therefore, ***a total of 53 pregnancies are required at EVMS. As treatments are associated with a 20% loss due to spontaneous abortion, or failure of neonate to thrive in an extrauterine environment, a total of 64 pregnancies (or 13 pregnancies/year over a 5-year period) are required to complete the objectives outlined.*** Based on our experience and multiple use of baboons (control, estrogen suppression, estrogen treatment etc), a population of 20 adult female and 3 adult male baboons (proven breeders) is required to meet the objectives of this study. Because multiple surgeries are limited, we also have determined that we need to purchase at least 3 and up to 5 adult female baboons yearly to "turn over" the colony.

SURGERIES/PROCEDURES IN ADULT PREGNANT ANIMALS:

HUSBANDRY:

All baboons are housed in USDA regulated cages. Socialization and behavior is monitored by PI staff and CompMed jointly. When possible, female baboons are socialized and pair housed with compatible females. Some pairs are fully open allowing free interaction. In some cases as a result of aggressive behavior causing injury or other negative physical conditions, two females are 'partial paired' meaning they do not have continues free interaction but are restricted allowing tactile contact and socialization on a limited level. There are some animals which cannot successfully be paired to any level. All animals are housed in rooms with multiple other animals allowing for vocal and visual stimuli. Socialization records are documented and kept by CompMed. Cycling adult female baboons are paired with male baboons for breeding purposes 5 days prior to ovulation as determined by perineal turgescence or sex skin swelling. Pregnancy is confirmed by ultrasound on day 25 (day 0 = day of ovulation; perineal detumescence) and/or failure to menstruate and absence of sexual skin swelling.

Blood Sampling: Animals are sedated with ketamine (10-15mg/kg IM). Blood samples are taken from the femoral or saphenous vein at 2-4 intervals dependent on treatment group. The area is cleaned with alcohol and blood samples (3-5 ml) obtained using 23g-21g needle for determination of blood chemistries (e.g. Na, K, glucose) and hormone profiles (cortisol, androgens, estradiol; insulin, prolactin, growth hormone, ACTH). Blood chemistry will be checked monthly using in house iSTAT analyzer. Animals will be weighed once a month when on study. The total blood sample volume < 10% total blood volume/not to exceed 10 ml/kg/month. Animals are returned to home cage and monitored for recovery.

Intravenous glucose tolerance test (ivGTT) at early and late gestation with amniocentesis at early gestation: An ivGTT will be performed at early (~80-120d) and late (~140-160d) gestation. Baboons (14kg -20kg body weight) are fasted overnight, and sedated with ketamine, intubated and O_2 delivered. Back of both legs are shaved for catheter placement. Anesthesia is achieved via IV into an antecubital vein and a constant infusion of ketamine (0.1 mg/kg) and propofol (0.2 mg/kg) 0.6ml/minute. Once sedated, two base line blood samples are collected and blood glucose and blood chemistries/gas levels determined using IStat. Approximately three minutes later, at experimental time zero, a bolus of glucose (0.25 grams/kg BW) is injected over a 10-15 second period into the antecubital vein. Blood samples (total blood sample volume < 10% total blood volume/not to exceed 10 ml/kg/month) are collected from the saphenous vein catheter at 1, 3, 5, 10, 20, and 30 mins. During the experiment, the animal is constantly monitored for BP, HR, respiration, and warmed via warming blanket. At early gestation, after final blood sample is taken, fetal/placental position is determined using GE Logic+ ultrasound. The abdomen is cleaned with alcohol and an 18g x 2in needle inserted through the uterine wall into the amniotic cavity and 10 ml collected. Fetal HR is rechecked. This portion of the procedure takes less than 10 minutes. At completion of the experiment, animal is given 1cc (10mg/kg) iron dextran IM. Catheters removed and animal will be monitored for swallowing reflex and response to stimuli before extubated. Animals are then returned to their cages. Completing iv GTT and amniocentesis concurrently reduces the number of sedations.

Brachial arterial flow mediated dilation (FMD) following shear stress by Doppler ultrasound. A non-invasive FMD will be performed at early (~80-120d) and late (~140-160d) gestation in adult pregnant. Briefly, baboons are fasted overnight, and sedated with ketamine. Animals are intubated and O2 is delivered. Anesthesia is achieved via IV into an antecubital vein and a constant infusion of ketamine (0.1 mg/kg) and propofol (0.2 mg/kg) 0.6ml/minute. Doppler flow analysis of brachial artery diameter and flow determined before and after induction of shear stress. During the experiment, the animal is constantly monitored for BP, HR, respiration, and warmed via warming blanket. Baseline measurements of the brachial artery are taken once stable plane is established (BP and HR stabilize). Doppler flow analysis of brachial artery diameter and flow will then be determined over a 5 minute period (7 measurements). To induce stress response, a blood pressure cuff is placed distal to the brachial artery (wrist) and pressure increased to 50 mmHg above systolic pressure for 5 minutes to occlude flow. The cuff is then released which induces shear stress (increased flow) which should induce endothelial cell nitric oxide production and cause vasodilation. Fetal HR is checked at the start and finish of the experiment via ultrasound. Once measurements are complete, anesthesia will stop and the animal will be monitored for swallowing reflex and response to stimuli, extubated and returned to the home cage to be monitored until upright. Blood chemistry is evaluated at the start or completion of the experiment.

Doppler analysis of utero-fetal-placental blood flow and response to serotonin at term (day ~160-170): As indicated in discussion of Study I, uterine artey remodeling is suppressed in baboons administered estradiol (25 µg/kg maternal body wt) daily on days 25-59 of gestation and have proposed that the latter impacts utero-placental blood flow dynamics later in gestation. To test this hypothesis, we propose to determine basal (resting) and serotonin-induced uterine arterial and umbilical (fetal) arterial and fetal middle cerebral arterial blood flow dynamics as well as fetal heart rate using 2D Doppler ultrasound during late gestation in pregnant baboons untreated or treated on days 25-59 with estradiol (25 µg/kg; Study I). Baboons are sedated with ketamine, and a catheter inserted into a peripheral saphenous vein and into an antecubital vein and a constant infusion via the saphenous vein of ketamine (0.1 mg/kg) and propofol (0.2 mg/kg)/0.25 ml saline/minute initiated and animals monitored for BP, HR, respiration, and warmed via warming blanket. A baseline blood sample (3-5ml) is obtained IV catheter to determine blood chemistries, gases and acid/base status and subsequent analysis of estradiol, progesterone and androgens. Animals are infused with saline (0.5 ml/min) for 20 minutes and fetal heart rate measured/monitored and uterine, umbilical, and fetal middle cerebral arterial blood flow dynamics determined during the final 5 minutes of infusion using 2D Doppler ultrasound. After collection of baseline data, a maternal infusion of serotonin (4 µg/kg/min) is initiated and fetal heart continuously measured and blood flow/ chemistry studies performed during the final 5 mins of this 20 minute infusion. Upon completion, the dose of serotonin is increased to 8 µg/kg BW/min and blood flow/chemistry analyses determined as described. Infusion of serotonin will be stopped immediately should fetal heart rate decrease to 80 bpm. If fetal HR stays below 80 bpm for more than 3 minutes, terbutaline will be administered IV/SQ to the mother under direction of the attending veterinarian to alleviate the fetal bradycardia. If fetal HR does not return to normal and continues to drop or fetal demise appears imminent, a cesarean section will be performed after consulting with the AV as described in our protocol. Fetal HR is checked at the start and finish of the experiment via ultrasound. At completion of the experiment catheters removed and animal will be monitored for swallowing reflex and response to stimuli before extubated. Animals are then returned to their cages.

Cesarean section: On days ~60 to ~170 of gestation based on the study group, baboons are sedated with ketamine (10-15 mg/kg), intubated, intubated and anesthetized with isoflurane/oxygen and vitals (e.g. HR, BP, CO2, RR, and temperature) monitored by CompMed staff. A catheter is placed in the antecubital/brachial vein and IV fluids administered. A second catheter is placed in the saphenous vein for blood sampling using a 19g catheter 24inches in length and IV fluids administered (1.6 ml/min over a 90 min period). The animal's abdomen/surgical site is shaved and scrubbed with alcohol and Betadine solution. The animal is draped using sterile technique. Blood samples (3-5ml) are obtained from the mother at '0' time, mid procedure and post placental delivery via saphenous catheter. Prior to surgery, a proper plane of anesthesia will be assured by lack of response to a noxious stimulus such as toe pinch. A vertical mid-line incision is made using a 10 blade. The incision is 4-24cm in length depending on gestational age. Once the abdomen is open, the uterus is externalized and examined for placenta location. Mother's ovaries are examined for evidence of ovulation/corpus luteum.

Two to four uterine vein blood samples are taken (5 ml) using a 23g needle. Warm, sterile fluids are applied to the uterus as needed. Fetal crown is localized and a horizontal incision is made in the uterus to remove the fetus. A sample of amniotic fluid is recovered using a syringe. Once the fetus is carefully exteriorized, umbilical vein and artery samples are taken using a 23g butterfly set (4-8 ml) for hormone, metabolic and blood gas analyses. The umbilical cord is double clamped to ensure the safety of the mother. At this point, one of four procedures will follow: 1) fetus is euthanized and mother recovers; 2) fetus and mother are euthanized; 3) fetus and mother survive; 4) fetus survives and mother is euthanized. Details of each procedure will be outlined in separate Attachment E forms.

SURGERIES/PROCEDURES IN ALL EVMS BORN NEONATES:

Blood Sampling: Babies are examined daily by Dr. Pepe's staff and weaned from their mothers at 8-12 months of age. At 2-4 week intervals beginning at approximately 6 months of neonatal age, mothers are sedated with ketamine and all neonates removed, sedated with ketamine, weighed, a gross physical examination performed by PI staff and blood samples (3 ml) obtained using 23g-21g needle for determination of blood chemistries (e.g. Na, K, glucose) and hormone profiles (cortisol, androgens, estradiol; insulin, prolactin, growth hormone, ACTH). The following studies will be performed prior to and after onset of puberty. (total blood sample volume < 10% total blood volume/not to exceed 10 ml/kg/month) Animals are returned to home cage and monitored for recovery.

Intravenous glucose tolerance tests with muscle biopsy T'0'&'30': an ivGTT will be performed prior to puberty at ~24-30 months and at one-two year intervals after puberty into adulthood (to age 15 years). Briefly, baboons are fasted overnight, and sedated with ketamine. Animals are intubated and O2 is delivered. Back of both legs are shaved for catheter placement. Anesthesia is achieved via IV into an antecubital vein and a constant infusion of ketamine (0.1 mg/kg) and propofol (0.2 mg/kg) 0.6ml/minute. Once sedated, two base line blood samples are collected and blood glucose and blood chemistries/gas levels determined using IStat. Approximately three minutes later, at experimental time zero, a bolus of glucose (0.25 grams/kg BW) is injected over a 10-15 second period into the antecubital vein. Blood samples (total blood sample volume < 10% total blood volume/not to exceed 10 ml/kg/month) are collected from the saphenous vein catheter at 1, 3, 5, 10, 20, and 30 mins. During the experiment, the animal is constantly monitored for BP, HR, respiration, and warmed via warming blanket. In the ivGTT performed at 24-30 months of age and in one ivGTT performed post-pubertal year of age, a biopsy of skeletal muscle (vastus lateralis) will be obtained prior to (zero time) and 30 minutes after injection of glucose which will cause a rise in insulin levels and allow us to determine responsivity of skeletal muscle (e.g. expression of insulin signaling molecules; metabolic enzymes as determined by Western blot/RT-PCR) and relate findings to insulin sensitivity/resistance as determined by the ivGTT. *See Attachment E for complete biopsy description*. At completion of the experiment, animal is given 1cc (10mg/kg) iron dextran IM. Catheters removed and animal will be monitored for swallowing reflex and response to stimuli before extubated. Animals are then returned to their cages.

Terminal IVGTT with muscle biopsy T'0'&'30' For those animals that have completed the study of the role of estrogen in pregnancy, a terminal study will be done as outlined above. Once second biopsy has been taken, the animal will be euthanized via IV injection of Beuthansia-D solution. Once death is confirmed with absence of heartbeat, tissues will be harvested as listed later.

Brachial arterial flow mediated dilation (FMD) following shear stress by Doppler ultrasound: non-invasive FMD will be performed prior to puberty at ~24-30 months and at one-two year intervals after puberty into adulthood (to age 15 years). Briefly, baboons are fasted overnight, and sedated with ketamine. Animals are intubated and O2 is delivered. Anesthesia is achieved via IV into an antecubital vein and a constant infusion of ketamine (0.1 mg/kg) and propofol (0.2 mg/kg) 0.6ml/minute. Doppler flow analysis of brachial artery diameter and flow determined before and after induction of shear stress. During the experiment, the animal is constantly monitored for BP, HR, respiration, and warmed via warming blanket. Baseline measurements of the brachial artery are taken once stable plane is established (BP and HR stabilize). Doppler flow analysis of brachial artery diameter and flow will then be determined over a 5 minute period (7 measurements). To induce stress response, a blood pressure cuff is placed distal to the brachial artery (wrist) and pressure increased to 50 mmHg above systolic pressure for 5 minutes to occlude flow. The cuff is then release which induces shear stress (increased flow) which should induce endothelial cell nitric oxide production and cause vasodilation. This study will allow us to ascertain whether endothelial function is compromised and thus programmed in utero. Once measurements are complete, anesthesia will stop and the animal will be monitored for swallowing reflex and response to stimuli, extubated and returned to the home cage to be monitored until upright. Blood chemistry is evaluated at the start or completion of the experiment.

Brachial artery flow by 2D Doppler and Microvascular flow by contrast enhanced ultrasound/microbubble (CEU/MB) Doppler: An experiment is performed prior to puberty at ~24-30 months and repeated in the post pubertal period at 6-12 years of age. Briefly, baboons are fasted overnight, and sedated with ketamine. Animals are intubated and O_2 is delivered. Anesthesia is achieved via IV into an antecubital vein and a constant infusion of ketamine (0.1 mg/kg) and propofol (0.2 mg/kg) 0.6ml/minute. During the experiment, the animal is constantly monitored for BP, HR, respiration, and warmed via warming blanket. 3-5ml blood is collected via saphenous or femoral vein for analysis of blood chemistries, acid/base and gases (I STAT), saline is infused (0.2 ml/min) via the antecubital vein and Doppler analysis of brachial artery diameter (right or left arm) and flow then determined over a 5 minute period (~7 measurements). Sterile lipid-encapsulated perfluorocarbon gas 2 µm diameter MB contrast agent (4 x 10^8/kg BW) will then be infused via the antecubital vein (1.0 ml/min). Bubbles are recognized by the 6.0-2.0 MHz 6C2 curvilinear transducer/CEU probe stabilized

over an area of the alternate arm and regions of interest identified on the computer. The MB are then broken by increasing the mechanical index from 0.2 to 1.0 for 2 seconds. The rate of MB contrast agent replenishment is then determined over the subsequent 60 seconds (all computerized; image capture = 1 frame/200 msec) and rate of refilling, slope and time to refill reflect flow velocity and the plateau achieved reflects total blood volume. Values are generated by computerized Axium Auto Tracking Contrast Quantification software. The increase in mechanical index (noise) to 1.0 does not harm vessels or cause sonoporation which requires increasing the mechanical index to 1.9. Baboons will then receive an infusion of pharmaceutical grade phenylephrine at 1 and 5 µg/kg bw/min/0.3 ml saline or of pharmaceutical grade nitroprusside at 1 and 3 µg/kg bw/min/0.3 ml saline, or of chemical grade acetylcholine (4 and then 8 µg/kg bw/min/0.3 ml saline before and during concomitant infusion of chemical grade N-nitro-L-arginine methyl ester (L-NAME; 40 µg/kg bw/min) for 5-7 min per dose to permit the brachial flow and measurements. The maximum dose of phenylephrine (5 µg/kg bw/min) proposed in the baboon is well within the range (10 µg/kg bw/min) that is administered to humans and which does not elicit excessive hypertension or tachycardia. The maximum doses of nitroprusside (3 µg/kg bw/min) and acetylcholine (8 µg/kg bw/min) proposed in the baboon, are the average dose infused to humans and which do not cause excessive hypotension, bradycardia or cyanide toxicity (NIH Daily Med Search; Medicine Online; Reed et al., Am J Physiol E472, 2004). L-NAME (Chemical grade) has been infused in human studies at a dose of 67/µg/kg bw and elicited no untoward effects (Jones et al, J Physiol, 560:329, 2004). Pharmaceutical grade L-NAME is not available and chemical grade as used in human studies will be employed in proposed experiments. At conclusion of the infusion of vasoactive agents, another blood sample (3-5 ml) will be obtained to verify blood chemistries, gases and acid/base status of animals. Infusion of octafluoropropane gas-filled albumin microbubbles and CEU have been used to assess microvessel flow in nonpregnant humans with no adverse effects (Timmerman et al., JCEM l95:3848, 2010; Lindner et al., J Am College Cardiac Imaging 1:343, 2008). Although vasoactive agents are not expected to induce any major change in vascular/respiratory status, should *a continued elevation or depression in BP or HR or respiration occur during infusion of any dose of any of the vasoactive agents, the infusion will be stopped and if needed, corrective action taken under the direction of the veterinarian.* At least one month later the analyses are repeated but phenylephrine (or nitroprusside or acetylcholine ± L-NAME) infused such that each animal receives sequentially all 3 agents in a randomized manner. In addition, prior to infusion of the second agent we propose to collect a biopsy of skeletal muscle for histologic/biochemical analyses. The entire protocol is then repeated in the post pubertal period (at 6-12 years of age). Once measurements are complete, anesthesia will stop and the animal will be monitored for swallowing reflex and response to stimuli, extubated and returned to the home cage to be monitored until upright. *See Attachment E for complete biopsy description.*

14. Adverse Effects: Monitoring and Management:

14a. In detail, describe the possible adverse effects for each experimental procedure and/or agent administered to the animals. For each item, include a statement detailing how the adverse effects will be clinically managed, should they occur.

Ketamine: IM injection for chemical restraint prior to all procedures including blood sampling. Ketamine is a dissociative anesthetic. Animals can develop tolerance and require increasing doses for effective sedation. Adverse effects can include nerve damage (if injection is improperly placed) and decreased appetite. Also, Ketamine can have a long term effect on kidney function. When possible, the lowest dose is used and each animal is evaluated on its responsive behavior to the drug. All changes in weight, appetite or blood chemistry are reported to veterinary staff (veterinary technicians and/or veterinarian).

Ketamine: Propofol (Ketafol): IV infusion for all procedures EXCEPT cesarean section. Potential adverse effects of ketamine are listed above. Propofol is very much used in humans and thus no major adverse effects are anticipated. However, propofol at high doses can depress blood pressure as well as compromise oxygen saturation and respiratory rate. Although we do not anticipate needing to increase propofol dosage to maintain sedation, we will intubate baboons and provide oxygen (to maintain 100% saturation) in all baboons in which Ketafol (ketamine:propofol) anesthesia is employed.

Flunixin meglumine (Banamine): Banamine will be injected IM for pain management. This may cause GI upset if given for too long or overdose. If GI upset is observed (loss of appetite) an alternate medication will be given.

Isoflurane: inhaled to maintain proper plane of anesthesia during all cesarean section (intraabdominal) surgical procedures. Adverse effects: none anticipated. Animals are closely monitored during procedures. If the animal moves, shows eye movement, has increased jaw tone, or shows a rapid increase in heart rate or blood pressure, then isoflurane administration will be increased. Possible side effects can be hypotension, dose-dependent respiratory suppression, cardio depression and GI effects (nausea, vomiting, ileus).If animal shows a decreased heart rate, decreased blood pressure, or pale gum color with reduced capillary refill time (CRT), then isoflurane administration will be decreased, along with decreased intravenous fluid flow rate.

Ketoprofen: PO administration for pain relief as an alternative to IM Fluxin meglumine. Long term administration can cause ulceration of the GI tract and GI bleeding; more rarely kidney damage and other bleeding disorders can occur. Adverse effects are not anticipated with the short-term administration described in this application.

Abdominal surgery: general risks associated with abdominal surgery include blood loss, infection, and adhesions. Undetected blood loss will be prevented by ensuring hemostasis before closing surgical incisions. All animals are monitored during the post-operative period (as defined by IACUC policy) for signs of internal bleeding (vasoconstriction and resulting loss of color of digits/extremities, lethargy, dehydration). Infection will be minimized by use of sterile equipment and

supplies, disinfection of the incision site, performance of surgery in a dedicated surgical suite, and use of aseptic technique during the procedure. Infection rate is minimal to none in 300+ survival surgical procedures performed by the PI/PI staff at EVMS. The Veterinarian will be consulted if unusual redness, swelling, or discharge is noted at the incision site. Adhesions will be minimized by gentle manipulation of internal organs and lavage of the abdominal cavity with warm saline to remove clotted blood before closing surgical incisions.

Muscle biopsy: minor procedure. Some possible complications may include bruising and discomfort at the site and infection at the site. Reduced movement may be seen for a short period. Analgesia is given to prevent discomfort.

Amniocentesis: general side effects are stress to the fetus and possible abortion. A clear area free of fetus and placenta is localized and a sample (10ml) of amniotic fluid removed. Fetal HR is checked. Any distress noted will be discussed with the Veterinarian.

IVGTT: side effects are minimal. Possible short term anemia and depreciated appetite from sedation. Animals are given Iron Dextran injection at completion of experiment and supplemented with children's vitamin containing iron. Also, food intake following experiment is monitored.

Dextrose (50%): local pain and vein irritation may occur. Diabetic coma, delirium tremors and congested states or pulmonary edema are unlikely but potential consequence. HR and BP are monitored before and after injection. Fetal HR is checked in the case of pregnant baboons.

Sodium Nitroprusside: relaxation of vascular smooth muscle and consequent dilation of peripheral arteries and veins. Change in BP could occur. If there is a consistent increase or decrease in HR or BP the infusion will be stopped and the veterinarian contacted for treatment options.

Phenylephrine: irregular heart rate, respiratory changes, allergic rash. HR, BP and body temp are measured throughout the experiment. If there is a consistent increase or decrease in HR or BP the infusion will be stopped and the veterinarian contacted for treatment options. At this low dose, we do not anticipate any problems or changes in animal behavior.

Acetylcholine: an endothelial cell dilator and thus a vasodilator of peripheral arteries and veins; at the maximal dose used, we do not anticipate severe hypotension or bradycardia; however, if the latter are pronounced or mean arterial BP drops below 40 mm Hg the infusion will be stopped and the veterinarian contacted for treatment options

N-nitro-L-arginine methyl ester (L-NAMME) This drug blocks the vasodilatory effects of acetylcholine (i.e. blocks endothelial cell nitric oxide production) and thus could cause hypertension and decrease heart rate. However, this is unlikely at the doses employed. The drug has been used in human studies at doses 1.5 times greater than in proposed experiments without causing any significant change in BP or HR. The drug is not available in pharmaceutical grade and chemical grade has been used in humans.

Serotonin: at this low dose, we do anticipate any change in behavior or long term physiological effects.

Octafluoropropane gas-filled albumin microbubble suspension (MB contrast): No expected adverse effects.

Letrozole: Administration of Letrozole alone (i.e. without concomitant administration of estradiol) lowers estrogen levels by >95%. When Letrozole treatment is initiated on day 100 and estrogen suppressed, approximately 10% of the baboons abort without any complications (vaginal bleeding visible); the products of conception may or may not be visible in the cage. In this case, the study is terminated, the animal watched closely over the next few days to ensure that bleeding has stopped, appetite is not depreciated and behavior is normal. In another subset of animals (approximately 10%), there is a potential for sudden onset of seizures at approximately day 120-150 gestation (i.e. approximately 20-50 days of letrozole treatment. Animals are typically found lying down (comatose) in their cage early in the morning suggesting that seizure(s) most likely occurred overnight or very early that morning. In animals that have seized, we believe it is important to intervene at time of discovery since it is our impression that the longer the animal is left comatose, the more difficult it is to revive the animal. The following protocol seems most relevant to implement and to have been a success in the past:

- Animals which are stuporous (unsteady on their feet but conscious) will immediately be given oral juice/sugar treatment in form of frozen juice or piece of orange or candy to elevate blood glucose levels. If animal is non responsive or progresses to seizing or unconscious state the following will be implemented:
- The animal is removed from its cage and taken to the treatment room. If light sedation is required for safe transport, a small dose (5 mg/kg BW-IM) of ketamine will be administered.
- Blood gas (pO_2, $pC0_2$, pH etc) and glucose will be determined using I-Stat analyzer (results in 2 mins).
- Animal will be placed on O_2 at 2L/min via cone mask and body temperature recorded and maintained with warming blanket and warm IV fluids.
- A catheter will be placed in an antecubital or saphenous vein and if blood glucose levels are below 50 mg/100 ml, a 5 ml bolus of 50% Dextrose in Lactated Ringers (1:1) will be delivered over a 5 min period, followed by a 5% Dextrose drip until the animal responds (glucose normalized). Adjustments to normalize pH (e.g. sodium bicarbonate) may also be required.
- Once responsive, the animal is returned to its cage and monitored throughout the day.
- The animal will be removed from the protocol and will most likely abort. If the latter does not occur, the animal will be permitted to go to term and the fetus delivered by cesarean section at the end of treatment.

It is important to note that of the 10% that do seize, approximately 35% succumb. It is our impression that these are most likely the animals that exhibited a seizure during the night. We have identified an apparent window between 120-140 days gestation when the seizures are most likely. We (PI staff) have been evaluating changes in glucose levels and blood gases as a way to determine if the seizures will occur. Comp Med personnel, as well as my staff, are aware of this possible linkage

and are attempting to resolve this problem. We do want to point out that regardless of when an animal seized, we still will employ the protocol outlined above (i.e. response to question 15a). In addition; decreased appetite can be seen during late treatment with Letrozole. Animal's gums can become swollen making hard biscuit consumption difficult. In this case, affected animals are given biscuits softened soaked in Ensure or Boost to assist in palatability and ensure necessary nourishment. If an animal continues to exhibit inappetence despite this treatment or experiences weight loss, the veterinarian will be consulted.

We do want to point out that pregnant baboons treated with letrozole and estradiol-benzoate do NOT exhibit seizures.

Estradiol-17β-3-benzoate: SC injections; no adverse side effects anticipated; irritation of skin at the injection site could occur but has not been noted in previous studies.

Blood sampling: A potential problem is increased ketamine tolerance, anemia/low hematocrit and sensitivity at injection site. Ketamine tolerance will be managed by using the lowest dose possible for the procedure. We will monitor hematocrit by taking a blood gas reading bi-weekly from animals on study. Vitamin supplements will be given. If prolonged anemia is seen, the Veterinarian will be consulted. To reduce sensitivity at the injection site, when possible the animal will be injected at different sites on the rump or large leg muscle area. In addition to ensure that animals health is not compromised, blood draw will not exceed 10% of the circulating blood volume or 10ml/kg/month.

14b. Describe the clinical parameters to be monitored to indicate adverse effects, pain, and/or distress to the animals. The parameters should be specific to the species and to the procedure(s) performed. Include the frequency of monitoring throughout the study.

Animals in the baboon colony are checked by PI staff and twice daily by CompMed staff. All staff will determine if each animal is eating, urinating, passing stool, and demonstrating the repertoire of behaviors normal for the individual animal. Immediately after surgery and during the postoperative period as defined by IACUC policy, animals will be observed daily by PI staff, with these observations recorded on postoperative evaluation sheets which become part of the animals' permanent records. Postoperative evaluation will include specific assessment of pain; failure to eat or decreased appetite, drink, urination or fecal output, change in normal repertoire of behaviors, may indicate pain. Lethargy and guarding of the incision site(s) may also indicate pain. If any of the above is seen during the postoperative monitoring period, the Veterinarian will be notified. Animal weight is monitored while on study protocol weekly when sedated. Blood chemistry is ran monthly as well. Any changes are relayed to the AV and determination followed.

14c. What conditions and/or complications will lead to removal of an animal from the study (*i.e., early endpoint parameters*)?

Animals will be considered for euthanasia as described in the IACUC protocol entitled "Guidelines for Early Removal Criteria and the Use of Death as an Experimental Endpoint". In addition, the Veterinarian may remove an animal from a protocol if a significant health problem is identified.

G. ADMINISTRATION OF ANESTHESIA, THERAPEUTICS, AND EXPERIMENTAL AGENTS:

15. Indicate the sedatives/tranquilizers, anesthetics, analgesics, antibiotics, and other relief agents to be administered. If no anesthetics, analgesics, or other pain relief methods will be used, please provide a strong justification for withholding analgesic agents in Question 15a. Justification for withholding analgesic agents must be based upon cited scientific fact or provided experimental data.

Some anesthetics, analgesics, tranquilizers, sedatives, or hypnotic agents are controlled substances and require Virginia Board of Pharmacy and DEA licenses for purchase and use. The DEA defines a controlled substance as *any substance listed in the Controlled Substances Act, Code of Federal or Substances Regulations* (21 CFR, part 1300 to the end). The DEA requires a research license for use of Schedule I-V agents in animals.

Does this project involve the use of one or more DEA regulated controlled substances? (http://www.deadiversion.usdoj.gov/schedules).

☒ YES

☐ NO

Name of the Controlled Substance	DEA Schedule #	Intended Use (e.g., analgesia, euthanasia, etc.)	Dosage
Ketamine HCl	II	Anesthetic/sedation	10-15mg/kg and 0.1mg/kg (with propofol)
B-euthanasia solution	II	Euthanasia	1mg/5kg

Name of the DEA Registrant	*Gerald J. Pepe Ph.D.*
DEA Research License Registration Number or Application Confirmation Date	[redacted]
DEA License Effective Date	*07/08/2014*
DEA License Expiration Date	*08/31/2017*

UNAUTHORIZED USE OF A DEA REGULATED SUBSTANCE MAY RESULT IN SUSPENSION OF THE IACUC-APPROVED PROTOCOL.

ADD ADDITIONAL ROWS AS NEEDED.

	Dose (mg/kg)	Route	Frequency of Administration
Sedatives/Tranquilizers			
Anesthetics: General			
ketamine-HCl	10-15 mg/kg	IM	Chemical restraint for all protocol blood sampling (1-4 days a week), ultrasound exam, or preoperatively
Ketofol: ketamine HCl (0.1mg/kg body weight: propofol (0.2 mg/kg body weight in 0.9% Sodium Chloride	Infused iv to deliver 0.1 mg ketamine: 0.2 mg/ propofol/minute/0.3 ml saline	IV	Chemical restraint for all procedures EXCEPT cesarean section including IVGTT, flow mediated dilation/brachial artery, skeletal muscle biopsies; micro-vascular flow by contrast enhanced ultrasound microbubbles
Isoflurane gas	~1-3% for maintenance	Inhaled	All cesarean sections, fetal injection
Anesthetics: Local			

Analgesics	Dose (mg/kg)	Route	Frequency of Administration	Length of Administration
Flunixin meglumine (Banamine)	2mg/kg	IM	At surgery and IVGTT w/ biopsy and FMD w/ biopsy	Surgery + 2 days BID
Ketoprofen	75mg	PO	Post operatively for surgery and IVGTT w/ biopsy and FMD w/ biopsy	Alternative to IM injection when more suitable for the animal in question (2 days BID)
Antibiotics				
Miscellaneous Agents				
Terbutaline	Recommended dose: 0.25mg to be repeated 30 mins if no clinical change occurs	IV	PRN under vet consult in response to fetal HR depression due to pharmacological agent under FMD procedure	PRN (under AV consult)
intravenous fluids: 0.9% sodium chloride or similar solution for IV administration	approximately 10 ml/kg body weight per hour during surgery	IV	one dose intraoperative	one dose intraoperative
Iron Dextran	10 mg/kg, IM	IM	At completion of surgery, IVGTT study	once
Beuthanasia –D solution	1mg/5kg	IV	Once for termination	once

15a. **JUSTIFICATION FOR WITHHOLDING ANALGESIC AGENTS**

Analgesics will not be withheld.

16. Will agents other than anesthetics or analgesics (*e.g., drugs, reagents, cells, etc.*) be administered?

_X_ YES (Complete Question 17 for each agent. Add additional sections as needed) _____ NO (Skip to Question 18)

17. Agent: Letrozole

Agent Vehicle: Sesame oil (sterile)

Volume per Administration: 1.0-2.0 ml

Route of Administration: SQ
Site of Administration: Abdominal area or back
Frequency of Administration: Daily on days 100 to ~170 of gestation
List all expected side effects and/or changes in the animal's behavior: Depreciated appetite. Although the drug itself does not elicit any side effects, the fact that the consequence of drug therapy is a decrease in estrogen production/levels by >95%, we observe in 15%-20% of pregnancies premature delivery and/or maternal seizures. In instances where a mild seizure has occurred but animal has not become comatose, we stop drug treatment for 24-48 hours and monitor the animal. Drug treatment can then be re-initiated without further development of any problems. See section 14a.

17. Agent: Estradiol 17β – 3 benzoate
Agent Vehicle: Sesame oil (sterile)
Volume per Administration: 0.2 to 1.0 ml
Route of Administration: SQ
Site of Administration: Abdominal area or back
Frequency of Administration: Administered daily on days ~100-170 of gestation in conjunction with Letrozole to restore estrogen production. Also administered daily on days ~25-59 of gestation to baboons to prematurely elevate estrogen levels in early gestation
List all expected side effects and/or changes in the animal's behavior: None are anticipated

17 Agent: Serotonin (5-HT)
Agent Vehicle: 0.9% NaCl
Volume per Administration: 4 µg/kg BW/min/0.3 ml saline and then 8 µg/kg BW/min/0.3 ml saline (each for 20-30 min, i.e. in 'step-up' fashion)
Route of Administration: IV pump infusion
Site of Administration: Saphenous or brachial vein
Frequency of Administration: Once per pregnancy
List all expected side effects and/or changes in the animal's behavior: No expected adverse effects, Any observed change in behavior will be addressed with Veterinarian consult.

17. Agent: Octafluoropropane gas-filled microbubble suspension (MB contrast agent)
Agent Vehicle: % saline
Volume per Administration: 0.1ml/min ($4x10^8$ kg/bw)
Route of Administration: IV
Site of Administration: Saphenous or brachial vein
Frequency of Administration: At CEU Doppler study. Total of 16 administrations over the life span of the animal. Not to exceed once a month.
List all expected side effects and/or changes in the animal's behavior: None anticipated

17. Agent: Phenylephrine
Agent Vehicle: 0.9% saline
Volume per Administration: 1 µg/kg BW/min/0.3 ml saline and then 5 µg/kg BW/min/0.3 ml saline (each for 20-30 min, i.e. in 'step-up' fashion)
Route of Administration: IV
Site of Administration: Saphenous or brachial vein
Frequency of Administration: 4 times over the life span of the animal. Not to exceed once a month.
List all expected side effects and/or changes in the animal's behavior:
At this low dose, we do not anticipate changes in behavior or physiological function. HR, BP and body temp are measured throughout the experiment. If there is a consistent increase or unlikely decrease in HR or BP the infusion will be stopped and the veterinarian contacted for treatment options.

17. Agent: Sodium Nitroprusside
Agent Vehicle: 0.9% saline
Volume per Administration: 1 µg/kg BW/min/0.3 ml saline and then 3 µg/kg BW/min/0.3 ml saline (each for 20-30 min, i.e. in 'step-up' fashion)
Route of Administration: IV
Site of Administration: Saphenous or brachial vein
Frequency of Administration: 4 times over the life span of the animal. Not to exceed once a month.
List all expected side effects and/or changes in the animal's behavior:
At this low dose, we do not anticipate changes in behavior or physiological function. HR, BP and body temp are measured throughout the experiment. If there is a consistent increase or unlikely decrease in HR or BP the infusion will be stopped and the veterinarian contacted for treatment options.

17. Agent: Acetylcholine
Agent Vehicle: 0.9% saline
Volume per Administration: 4 µg/kg BW/min/0.3 ml saline and then 8 µg/kg BW/min/0.3 ml saline (each for 20-30 min, i.e. in 'step-up' fashion)
Route of Administration: IV
Site of Administration: Saphenous or brachial vein
Frequency of Administration: 4 times over the life span of the animal. Not to exceed once a month.
List all expected side effects and/or changes in the animal's behavior:
At this low dose, we do not anticipate changes in behavior or physiological function. HR, BP and body temp are measured throughout the experiment. If there is a consistent increase or unlikely decrease in HR or BP the infusion will be stopped and the veterinarian contacted for treatment options.

17. Agent: N-nitro-L-arginine methyl ester (L-NAMME)
Agent Vehicle: 0.9% saline
Volume per Administration: 40 µg/kg bw/min
Route of Administration: IV
Site of Administration: Saphenous or brachial vein
Frequency of Administration: 4 times over the life span of the animal. Not to exceed once a month.
List all expected side effects and/or changes in the animal's behavior. Could cause hypertension and decrease heart rate. However, this is unlikely at the doses employed as the drug has been used in human studies at doses 1.5 times greater than proposed without causing any significant change in BP or HR. The drug is not available in pharmaceutical grade and chemical grade has been used in humans.

Agent: Dextrose solution (50%)
Agent Vehicle: In solution
Volume per Administration: 0.25 grams/kg BW of 50% solution
Route of Administration: IV
Site of Administration: Saphenous or brachial vein
Frequency of Administration: Once at IVGTT
List all expected side effects and/or changes in the animal's behavior. local pain and vein irritation may occur. Diabetic coma, delirium tremors and congested states or pulmonary edema are unlikely but potential consequence. HR and BP are monitored before and after injection. Fetal HR is checked in the case of pregnant baboons.

17a. In accordance with the *Guide for the Care and Use of Laboratory Animals* (*Guide*, 8th edition), pharmaceutical-grade chemicals and other substances should be used for all animal-related procedures, when available (USDA 1977b). The use of non-pharmaceutical-grade chemicals or substances should be described and justified in the animal use protocol and must be approved by the IACUC (Wolff et al. 2003). The IACUC will consider ***exceptions*** to the *Guide* requirement for use of pharmaceutical-grade-chemicals in animal research studies when there is **"sufficient scientific justification" Please see the IACUC guidance document entitled, *Use of Non-Pharmaceutical-Grade Chemicals and Other Substances*, for examples of "sufficient scientific" criteria (http://info.evms.edu/Research_html/IACUC/Sample%_20SOPs.htm).**
If you wish to use non-pharmaceutical-grade drugs in the study, please provide your justification below

The drug L-NAMME is not available in pharmaceutical grade and chemical grade has been used in humans. Acetylcholine, estradiol-17β-3 benzoate, serotonin and phenylephrine are not available in Pharmaceutical grade. Available formulations either act at the receptor level (serotonin reuptake inhibitors) or are precursors of the drug (5-

hydroxytryptophan is converted to serotonin), are already dosed and in liquid to be used as an eye drop or inhalant (acetylcholine) or injectable (epinephrine) or in a solid/oral pill containing other additives such as di-calcium phosphate, cellulose, magnesium stearate, vitamin B6, and/or silicon dioxide etc. (steroid hormones e.g. estradiol). Chemical grade reagents are available and product information indicates preparations are very high grade >99% pure. Finally, we have used and were previously (IACUC #12-010) approved to use chemical grade phenylephrine, serotonin, estradiol17β-3 benzoate and acetylcholine in our pregnant baboons and/or their offspring with no apparent untoward or harmful effects.

NOTE: Your signature on page 5 certifies that all drugs used on animals before, during, or after an experimental or surgical procedure will be obtained from legal sources, will be pharmaceutical-grade, unless otherwise approved, and will be disposed of properly when out-of-date or no longer needed. All controlled substances MUST be kept in a double-locked compartment, and records documenting each use of a controlled substance MUST be maintained.

H. SPECIES SELECTION AND ORDERING:

16. Please indicate the species and the number of animals requested.

Species (Common Name & Strain)	Total # Requested for a 3-Year Period	Average # to be Maintained in the Animal Facility	Maximum # to be Maintained in the Animal Facility
Baboon (*Papio anubis/cynocephalus*)	206 (9 males for breeding,~9 spontaneously aborted fetuses, 64 used fetuses, 84 adult females and 40 juveniles)	35	50

If the protocol involves non-human primates, please complete Attachment B, NON-HUMAN PRIMATE ENHANCEMENT PROCEDURE.

19. Will animals be ordered through the Division of Comparative Medicine (CompMed)?

X YES _____ NO (Identify the source and provide the rationale/justification)

20. Will special housing be required (*e.g., specific bedding requirements, isolator cages, special feed or handling, etc.*)?

x YES (Describe all special requirements) _____ NO

The PI supports social housing of research primates. However, housing must allow PI/PI staff access to individual animals at all times for blood sampling, injection, and accurate postoperative evaluation.

Males are pair housed for 2-5 days with females during estrous for mating. Males are otherwise single housed due to elevated possible aggression toward unreceptive females.

Some adult females are incompatible with others and physical trauma arises, over grooming occurs and nutritional issues arise (one animal loses weight from not eating) or over submissive behavior. Other reasons for single housed animals are SIB which can be stressful to a cage mate.

Late gestation pregnant animals expected to deliver spontaneously will be single housed during late gestation to ensure safe delivery of fetus.

Most animals are at minimum partially paired allowing liberal touch but may be limited on full range contact. Semiannual reevaluation of the colony attempts to reintegrate those single housed animals. All animals will at a minimum have visual and auditory and olfactory sense of other NHP

21. Will animals be maintained as a colony over a long period of time?

(A colony is defined as "breeding or holding of animals for reuse in other experiments.")

X YES (Complete **Questions 21a and 21b**) _____ NO (Skip to **Question 22**)

21a. List the number of new animals to be purchased for the colony: 3

21b. List the number of animals to be used from an existing colony: 31

I. PERSONNEL TRAINING:

22.	In **Section 1**, list the name of each person involved with the project, along with the species to be used, the person's years of experience with that species, and the person's training information. In **Section 2**, note each person's functional role for each species listed.

SECTION 1: PERSONNEL INFORMATION

NAME:	Gerald Pepe	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
(Species / Experience)	Species / Exp	Species / Exp	Species / Exp	Species / Exp	Species / Exp	Species/Exp
Species used in project / Years of experience with the species listed	P anubis/39	P anubis/18	P. anubis/15	P Anubis/5	P. anubis/2	P anubis/ 2
	/	/	/	/	/	/
	/	/	/	/	/	/
Occupational Health and Safety (OHSP) Training Certification Number	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Occupational Health and Safety Risk Assessment Date (Month / Year)	4/21/15	4/22/15	3/8/14	8/8/14	2/5/15	2/5/15
CITI Training Certification Number and Species	89-039 Nonhuman primate	92-095 Nonhuman primate	00-004 Nonhuman primate	01-017 Nonhuman primate	08-113 Nonhuman primate	98-009 Nonhuman primate
SECTION 2: FUNCTIONAL ROLE FOR EACH SPECIES LISTED						
Supervision	X		X			
Care and Handling	X	X	X	X	X	
Anesthesia			X			
Surgery	X	X	X	X	X	X
Post-Surgical Care			X			
Monitoring	X	X	X	X		
Euthanasia	X		X			

NAME:		[redacted]				
(Species / Experience)		Species / Exp	Species / Exp	Species / Exp	Species / Exp	Species/Exp
Species used in project / Years of experience with the species listed	/	P anubis/2				/
	/	/	/	/	/	/
	/	/	/	/	/	/
Occupational Health and Safety (OHSP) Training Certification Number		[redacted]				
Occupational Health and Safety Risk Assessment Date (Month / Year)		5/7/15				
CITI Training Certification Number and Species		13-010 Nonhuman primate				
SECTION 2: FUNCTIONAL ROLE FOR EACH SPECIES LISTED						
Supervision						
Care and Handling		X				
Anesthesia						
Surgery		X				
Post-Surgical Care						
Monitoring						
Euthanasia						

22a. Provide information regarding the degree of training and procedural experience for each individual listed in Question 22.

Dr. Pepe has performed surgeries on baboons for more than 30 years. His team works closely with him to provide for collection of tissues during surgery. [redacted] has a wealth of experience working with these animals and is now an acknowledged expert in intubation/surgical preparation and performance of surgical procedures outlined in this protocol as well providing surgical assistance. Moreover, [redacted] has been trained by Dr. Pepe and has been performing surgeries (cesarean section) for 15 years without direct assistance of Dr. Pepe. Although Dr. Pepe has not scrubbed in he is available and now will routinely be on site to assist in collection of fetal tissues. [redacted] in consultation with Dr. Pepe actually performs all of the animal husbandry, e.g., animal injections; blood sampling etc and also working with CompMed and other laboratory personnel in hand-rearing of baboon neonates per SOP. [redacted] has had significant experience assisting Dr. Pepe in the conduct of the surgical experimentation and collecting tissue samples. She will perform many of the biochemical analyses outlined and assist in the preparation of fixative and all reagents used in these studies with the exception of Letrozole and analgesics. [redacted] has had over 10 years of experience with surgery in nonhuman primates and is available to assist should [redacted] or others be sick or unavailable. [redacted] is currently a postdoctoral fellow in Dr Pepe's laboratory and will assist in collection and performing biochemical analyses of baboon fetal tissues and biopsies of skeletal muscle from adolescent baboons as well as assisting [redacted] with conduct and analysis of the iv glucose tolerance tests and brachial artery flow mediated (FMD) studies and conduct of ultrasound studies of uteroplacental/fetal blood flow. [redacted] will be available to assist in collection of fetal and/or adolescent tissue biopsies as well as assist [redacted] with conduct of the iv glucose tolerance tests and brachial artery flow mediated (FMD) studies and conduct of ultrasound studies of uteroplacental/fetal blood flow. [redacted] will assist and provide advice on harvesting fat samples and preparation of appropriate fixatives for tissue preparation.

22b. List any person who will require supplemental training from CompMed and state the training required for each person.

None required

J. SURGICAL PROCEDURES:

ALL SURGICAL PROCEDURES MUST BE DETAILED IN ATTACHMENT E, ANIMAL SURGICAL PROCEDURES.

Surgery Classifications for All Vertebrate Animal Species

SURGERY TYPE DEFINITIONS	Type 0 Surgery	Type I Surgery	Type II Surgery	Type III Surgery
	Surgical procedures performed with appropriate anesthesia that do not require the use of additional analgesia	Surgical procedures that result in mild pain and require pre-emptive use of at least one dose of additional analgesia pre- or perioperatively	Surgical procedures that result in moderate pain and require pre-emptive use of at least one dose of pre- or perioperative additional analgesia lasting at least 24-48 hours	Surgical procedures or invasive manipulations that result in marked to severe pain and require pre-emptive use of at least one dose of pre- or perioperative additional analgesia lasting at least 72 hours
EXAMPLES OF PROCEDURES IN EACH SURGERY TYPE CLASSIFICATION	Small skin incision without deeper tissue manipulation; Skin biopsy; Placement of small subcutaneous implants; Tracheal injection; Retro-orbital bleed	Skin incision and deeper tissue manipulation, i.e., muscle incision, muscle biopsy, vessel cannulation (not percutaneous), vessel incision, vasectomy, non-extensive oral surgery and/or tissue manipulation, bone marrow aspiration	Laparotomy without organ manipulation or incision; Craniotomy (small burr hole); Placement of vascular access ports; Orchiectomy; Superficial lymph node biopsy; Thyroidectomy; Skin graft involving partial thickness and minimal body surface area	Laparotomy with significant organ manipulation or incision; Thoracotomy; Thymectomy; Orthopedic surgery to create or repair a bone fracture; Dissection into joints; Large craniotomy; Intraocular or corneal surgery; manipulation; Skin graft involving full thickness, multiple grafts, and/or extensive body surface area; Extensive oral or dental Surgery; Model involving extensive burns or tissue trauma; Parabiosis; Procedure involving infarction of organs with significant sensory innervation

Source Material: University of Michigan, University Committee on Use and Care of Animals, Policy on Analgesic Use in Animals Undergoing Surgery

K. ANIMAL USE PROCEDURES (EXCEPT SURGICAL PROCEDURES):

23. Will cells, tissues, and/or organs be collected?

X YES (Complete all applicable sections below) _____ NO (Skip to **Question 24**)

23a. Blood sampling

Technique: **[1] Pregnancy studies**: Blood samples (maternal) are collected at 2-4day intervals depending on the study group. Briefly, animals are restrained, injected with ketamine-HCl (10-15 mg/kg) and samples (3-5 ml) obtained from a saphenous or antecubital vein using a 21 gauge needle. **[2] Neonate-Adolescent studies**: Blood samples are obtained once every two weeks from neonates and prepubertal adolescents. Briefly, baboons are restrained, injected with ketamine HCl (10-15 mg/kg BW) and a 3 ml (neonates) sample obtained from a peripheral saphenous vein using a 21g needle. Weight is also recorded and monitored in this group. **[3] IVGTT Studies** 3ml Samples are collected into syringes via catheter at 00, 0, 1, 3, 5, 10, 20, and 30 and 0.1 ml examined for blood glucose and the remainder kept on ice and serum subsequently assayed for insulin/C-peptide. **[4] CEU / Doppler:** Two samples are taken, start of the experiment and completion of experiment, for blood chemistry and hormone analysis.

Sample site(s): Saphenous/antecubital/femoral

Volume per sample: Blood draw will not exceed 10% of the circulating blood volume or 10ml/kg/month for IVGTT, At surgery 3-5 ml but not to exceed 10ml/kg/month

Frequency and duration of sampling: 2-4day intervals during gestation from mother; once every other week from adolescents;

23b. Urine/feces sampling

Sampling method:

Frequency and duration of sampling:

23c. Collection of tissues

All tissues to be collected: Kidney, liver, lung, gonads, adrenal, pituitary, pancreas, skeletal muscle, visceral and SQ fat, intestine, heart and uterine samples.

When will tissues be collected (before or after euthanasia)? After euthanasia in both adult sacrificed animals and fetus following euthanasia at surgery

Final disposition of collected tissues: Fixed and/or frozen for experimentation

24. Will behavioral testing be conducted?

X	No behavioral testing will be conducted.
	Yes, behavioral testing will be conducted with significant restraint or noxious stimuli. (Complete **Attachment C, PROLONGED PHYSICAL RESTRAINT OR STRESS**)
	Yes, behavioral testing will be conducted without significant restraint or noxious stimuli. (Describe the procedure below)

25. Will a special diet be required?

_____ YES (Complete **Questions 25a-25c**) X NO (Skip to **Question 26**)

25a. Describe the anticipated nutritional deficit or supplementation.

25b. Provide the reason(s) for the supplementation and treatment of the deficit.

25c. How often will the animals be weighed? _____

How much weight change will be permitted before the animal will be removed from the study? _____

26. Will indwelling catheters or implants be used?

_____ YES (Complete a section below for each site. Add additional sections as needed) X NO (Skip to **Question 27**)

26a(1) Implant site: _____

Type and size: _____

Maintenance: _____ Duration: _____

26a(2) Implant site: _____

Type and size: _____

Maintenance: _____ Duration: _____

27. Will tumors be transplanted or induced?

_____ YES (Complete a section below for each site. Add more sections as needed) x NO (Skip to **Question 28**)

27a(1) Transplant or induction site: _____

Anticipated functional deficit(s) and management: _____

Maintenance: _____ Duration: _____

27a(2) Transplant or induction site: _____

Anticipated functional deficit(s) and management: _____

Maintenance: _____ Duration: _____

L. ANIMAL CARE:

28. Describe, in detail, the plan for medical care of the animals in the proposed study, and **identify, by name and job classification**, the investigative staff member(s) responsible for providing the care.

NOTE: Routine observation of the animals and medical intervention is the responsibility of the Principal Investigator.

All animals will be observed daily by [redacted] Animal Coordinator/Research Associate, and/or CompMed staff. Medical problems will be reported to the Veterinarian or a member of the CompMed staff. Postoperative monitoring will be performed for each animal after surgery as defined by IACUC policy. The animal's attitude (alert, responsive) is observed as well as the status of the surgical incision(s), food consumption, urine and feces production, and resumption of the animal's normal repertoire of behaviors; pain is also assessed as described. Postoperative observations are recorded on forms approved by CompMed, and these forms become part of the animal's permanent record

29. Will a special observation regimen be required?

X YES (Complete **Question 29a**) _____ NO (Skip to **Question 30**)

29a. Frequency of Observation: Once a day

By whom (Identify by Name and Title): [redacted], Research Associate II

Starting: Start of the study Ending: End of the study or euthanasia

[redacted] will be primarily responsible for all observations described above. Animals will also be observed to determine if menstruations have occurred. CompMed or trained member of the PI's staff will observe animals in [redacted] absence

30. Indicate any special instructions that should be observed for animals found dead (*e.g., call the investigator, refrigerate or freeze the carcass, dispose of the carcass, etc.*) **If you would like for the Attending Veterinarian to necropsy animals that die unexpectedly, please indicate how the tissues should be handled.**

Alert the investigator and for emergency animal care contact [redacted]. If necropsy is performed, collect uterus and adrenals, and pieces of kidney and liver (or others as determined by the Veterinarian) which are placed in fixative (4% paraformaldehyde or phosphate buffered formalin) for subsequent histopathology

M. DISPOSITION OF ANIMALS:

31. Please indicate the method(s) of animal disposition (**Check all that apply**)

X Euthanasia (Complete **Questions 33a-33c**)

_____ Death as an Endpoint (Complete **Attachment G, DEATH AS AN ENDPOINT**)

_____ Return to the animal colony

X Available for transfer to another EVMS IACUC-approved protocol*

X Available for transfer to another research institution*

_____ Available for adoption as a companion pet

_____ May be culled for tissue sharing

_____ Other (Explain)

* ***Animals that have undergone survival surgery in one IACUC protocol may not be transferred to another survival surgery protocol, unless the request is specifically reviewed and approved by the IACUC. Animals may be transferred to non-surgical or non-survival surgery protocols without IACUC review.***

32. Disposition of Surviving Animals

32a. Will animals survive the protocol procedures? If some animals will survive and others will not, please indicate both YES and NO and clearly state which animals will survive and which animals will not survive.

X YES (Complete **Question 32b**) X NO (Skip to **Question 33**)

Those animals that have met the allowable number of multiple surgeries will be terminated at the final surgery. Some animals may be removed prior to completion of 6 surgeries due to Health reasons all under the consult of the AV.

32b. Will animals survive without harm or disability? If some animals will survive without harm or disability and some will not, please indicate both YES and NO and clearly state which animals will survive without harm or ability and which animals will not survive without harm or disability.

X YES (Skip to **Question 33**) NO (Complete **Question 32c**)

32c. Describe the harm or disability and the plans for managing the condition(s).

33. Euthanasia

33a. Will the animals be euthanized?

X YES (Complete **Questions 33b-33d**) ___ NO (Skip to **Question 34**)

33b. Explain why the animals will be euthanized.

The maximal number of multiple survival surgeries has been achieved or the animal has developed problems either protocol related or clinical which compromise further surgical interventions or the experiment is a terminal procedure. We do attempt to relocate these animals and/or employ them as surrogate mothers for developing neonates. In addition, there is need to collect adult tissues (ovaries, adrenal etc) to serve as controls for our fetal and adolescent studies. In a small % of the colony, we are unable to achieve a pregnancy for reasons not clear to us (the animal does not have normal menstrual cycles, is not mensing at all or is not receptive to breeding). In this instance, we request that these few (<5%) animals be transferred to University of Maryland to be used in our study there.

33c. Indicate how the animals will be euthanized.

Euthanasia Agent/Procedure: Beuthanasia-D solution

Dose of administration: 1 mg/5kg

Route of administration: IV

33d. Per the AVMA (American Veterinary Medical Association) *Guidelines on Euthanasia*, most physical methods of euthanasia, when done appropriately, are "conditionally acceptable," meaning that the nature of the techniques may not consistently produce humane death or they present a greater potential for operator error or safety hazards. In those situations where physical methods may be the most appropriate method for euthanasia and rapid relief of pain and suffering, extreme care and caution must be exercised, and personnel performing physical methods of euthanasia must be well trained and monitored for each type of physical technique. **If a physical method, such as decapitation or cervical dislocation, will be used as the primary means of euthanasia, please provide scientific justification.**

N/A

N. ANIMALS BROUGHT INTO AND TAKEN OUTSIDE OF THE ANIMAL FACILITY:

34. Will animals be transferred into the CompMed animal facility from another institution?

X	YES (Complete Questions **34a-34b**)		NO (Skip to **Question 35**)
Name and location of the transferring institution:	University of Maryland School of Medicine (this is a rare occurrence and all EVMS policies are carried out and AV in consult)		

All animals received from other than approved vendors must undergo a quarantine period to allow for evaluation of the health status of the animals prior to their introduction into the colony. They may also require testing and segregation to determine their health status.

THE PRINCIPAL INVESTIGATOR SHOULD DISCUSS THIS MATTER WITH COMPMED PRIOR TO INITIATING ANIMAL TRANSFER.

THE PRINCIPAL INVESTIGATOR IS RESPONSIBLE FOR ALL RELATED CHARGES.

34a. How long will the quarantine or stabilization period last?

Three completed negative TB test results or as determined by the Institutional Veterinarian and the institution. 3-4 weeks minimum with the Tb tests and other undesired pathogens as per the AV

34b. How long will the animals be housed at EVMS?

Animals will be housed at EVMS until 1) the quarantine period is completed, 2) the assigned protocols are completed, and 3) reassignment within EVMS, transfer out of EVMS, or euthanasia. Generally, animals complete the assigned protocols within 1 year. Quarantine and preparation for disposition will add approximately 6 months. Unforeseen problems which disrupt the group may require additional months for stabilization of menstrual cycles before experiments can begin or continue.

35. Will the animals be taken out of the CompMed central animal facility for any reason (*e.g., manipulation, surgery, temporary housing, etc.*)?

_____ YES (Complete **Questions 35a-35c**) X NO (Skip to **Question 36**)

35a. To what building(s) and room(s) will the animals be taken? (Indicate the procedure(s) to be performed in each specific location (*e.g., surgery, examination, blood collection, euthanasia, etc.*))

35b. How will the animals be transported? (Be specific. Include all safety precautions for the animals and personnel.)

35c. How often will the animals be taken to the location(s) listed in Question 35a and for what duration of time per occurrence?

36. Will the animals be used or housed in locations outside of the CompMed central animal facility for more than 12 hours?

_____ YES (Complete **Questions 36a-36c**)* X NO

***The location must be certified as a satellite facility and undergo semi-annual inspection by the IACUC.**

36a. In what building(s) and room(s) will the animals be used or housed?

36b. Describe the animal husbandry to be performed and identify, by name and title, the person(s) who will provide husbandry.

36c. How long will the animals be used or housed in the satellite facility?

Eastern Virginia Medical School
Institutional Animal Care and Use Committee JUN 12 2015

Attachment B: Nonhuman Primate Enhancement Procedure

Project Title: Regulation of Fetal-Placental Development in the Primate

1. Paired housing: Nonhuman primates used under this protocol can be housed in the same primary enclosure with one or more compatible primates.

   X YES (*Skip to Question 2*) X NO (*Complete Question 1a*)

   1a. Justify why the animal must be singly housed: animals who are singled housed have demonstrated an inability to pair house. Injury to self or other animals, negative behavioral issues or consistent weight loss is also cause for single housing.

   The PI supports social housing of research primates. However, housing must allow PI/PI staff access to individual animals at all times for blood sampling, injection, and accurate postoperative evaluation.

   Males are pair housed for 2-5 days with females during estrous for mating. Males are otherwise single housed due to elevated possible aggression toward unreceptive females.
   Some adult females are incompatible with others and physical trauma arises, over grooming occurs and nutritional issues arise (one animal loses weight from not eating) or over submissive behavior. Other reasons for single housed animals are SIB which can be stressful to a cage mate.
   Late gestation pregnant animals expected to deliver spontaneously will be single housed during late gestation to ensure safe delivery of fetus.
   Most animals are at minimum partially parried allowing liberal touch but may be limited on full range contact. Semiannual reevaluation of the colony attempts to reintegrate those single housed animals. All animals will at a minimum have visual and auditory and olfactory sense of other NHP

2. Nonhuman primates used under this protocol will be provided with a variety of devices as described in the EVMS Primate Enhancement Program (this can be provided to you by the Office of Research or the Division of Comparative Medicine (CompMed) upon request).

   X YES _____ NO (*justify in the space below*):

Eastern Virginia Medical School
Institutional Animal Care and Use Committee

Attachment D: Use of Hazardous Agents

JUN 12 2015

Project Title: Regulation of Fetal-Placental Development in the Primate

This form applies to IACUC protocols using materials such as radioisotopes, infectious agents, carcinogens, biohazards, mutagens, teratogens, abortifacients, acutely toxic chemicals, or other potentially hazardous chemicals. Information provided in this form will enable assessment of risk to individuals handling or caring for animals, as well as risk to other animals. **If you need assistance answering any of the questions, please contact the Environmental Health and Safety Office at (757)** [redacted]

The Division of Comparative Medicine (CompMed) must be notified in writing at least one week prior to the use of hazardous agents in animals. The use of hazardous agents in animals is not permitted until approval has been granted by the CompMed Facility Manager. Please reference the CompMed SOP entitled, *Appropriate Notification, Signage and Protocol for Working with Hazardous Agents in Animal Holding Rooms and Procedure Rooms.*

YOU MUST COMPLETE A SEPARATE FORM FOR EACH HAZARDOUS AGENT USED.

1. Please indicate the type of hazardous agent being used. Check all that apply. For microorganisms, please list the Risk Group or Biosafety Level. For chemicals, please list the CAS # and the LD50 from the Material Safety Data Sheet (MSDS).

   ____ Radioactive material (Isotope: _____)
   ____ Infectious agent (Risk Group or Biosafety Level: _____)
   ____ Acutely toxic chemical (CAS #: _____, LD50: _____)
   ____ Known or suspected human carcinogen (Chemical Name: _____)
   __X__ Known or suspected human mutagen/teratogen (Chemical Name: Letrozole)
   ____ Recombinant DNA/RNA
   ____ Tissue sample (Type: _____)
   ____ Other (*Describe below*):

2. Please provide specific information about the agent:

   Complete name *(Include strain for microorganisms)*: Letrozole
   Dose and frequency of administration: Drug is prepared at a concentration of 2 mg/ml sesame oil; animals injected daily with 0.115 mg letrozole/kg BW/day (sesame oil volume ranges from 0.2 to 1.0 ml)
   Concentration: 2 mg/ml sesame oil
   Route: SC Duration of exposure: 10-60 days (determined by study)
   How long will the animal be maintained after administration? >3 years
   Animal species: *Papio anubis* Estimated animal weight: 14-18kg

3. Is the agent excreted or shed by the animal?

   ____ YES *(Indicate the type of excreta and estimated quantity per day)*
   __X__ NO

4. Are there documented human risks from exposure to the agent? (Risks may be determined from the MSDS or from references found in the Biosafety Manual.)

X YES *(Complete Questions 4a-4e)* _____ NO *(Skip to Question 5)*

4a. Indicate the route(s) of human exposure:

X Inhalation	X Contact
X Ingestion	_____ Parenteral
_____ Other (describe below):	

4b. Describe the risks associated with the agent (mutagen, teratogen, etc.):

Agent is known to suppress estrogen production in females .Letrozole is an oral, anti-estrogen drug that is used for treating postmenopausal women with breast cancer.

4c. Indicate the acutely toxic/infectious dose in humans (in mg/kg or number of organisms):

The standard dose of Letrozole used in women is 2.5 mg/day. We will be administering a maximum of 2 mg/day and do not anticipate any acute side-effects of the drug *per se*. Only PI staff is exposed to the agent

4d. Describe any genetic changes to the organism and their suspected effects:

N/A

4e. Describe the symptoms of exposure: Exposure would have to be long term and contact would need to be ingested or contact with an open wound. Basic exposure symptoms would be irritation or skin rash.

4f. Describe the first aid methods to be taken in the **case** of exposure:

Flush the area with water

4g. Indicate all personal protection required:

X Lab coat/dedicated clothing	_____ Apron
X Gloves	_____ Face shield
X Goggles	_____ Respirator
X Other (describe below): Mask	

5. Are there risks to other animals in the room or in the animal facility?

_____ YES *(Complete Questions 5a-5d)* X NO *(Skip to Question 6)*

5a. Describe the risk to other animals:

5b. Indicate the route of animal exposure:

5c. Describe all methods that will be used to contain the risk factor:

5d. Are special animal care requirements necessary?

_____ YES *(Describe below)* _____ NO

6. Are special waste or carcass disposal requirements necessary?

_____ YES *(Describe below)* X NO

7. Briefly describe the procedure(s) for administration of the agent and how the agent is metabolized and/or excreted. Include how long the agent is expected to remain in/be shed from the animal, the concentration of agent in any specific organs, etc.

7a. Please indicate whether or not the laboratory personnel and CompMed staff have been informed of these hazards and have received training on proper handling techniques.

Laboratory Personnel	**CompMed Staff**
X Yes	X Yes
_____ No	_____ No

If not, please contact the Environmental Health and Safety Office to schedule the proper training.

8. Please provide any other relevant information regarding the agent (e.g., unique containment recommendations): This agent is injected SC by PI staff and is not handled by Comp Med staff.

1. Please indicate the type of hazardous agent being used. Check all that apply. For microorganisms, please list the Risk Group or Biosafety Level. For chemicals, please list the CAS # and the LD50 from the Material Safety Data Sheet (MSDS).

____ Radioactive material (Isotope: _____)

____ Infectious agent (Risk Group or Biosafety Level: _____)

____ Acutely toxic chemical (CAS #: _____, LD50: _____)

____ Known or suspected human carcinogen (Chemical Name: _____)

____ Known or suspected human mutagen/teratogen (Chemical Name:)

____ Recombinant DNA/RNA

____ Tissue sample (Type: _____)

__x__ Other (*Describe below*):

2. Please provide specific information about the agent:

Complete name
(Include strain for microorganisms): Phenylephrine

Dose and frequency of administration: 1 µg/kg BW/min/0.3ml saline for 10-15 min then 5 µg kg BW/0.3ml saline for 10-15 minutes

Concentration: stock solution 1 mg per ml prepared day of experiment: _____

Route: IV Duration of exposure: < 60 minutes

How long will the animal be maintained after administration? >3 years

Animal species: Papio anubis Estimated animal weight: 4kg -18kg

3. Is the agent excreted or shed by the animal?

_____ YES *(Indicate the type of excreta and estimated quantity per day)*

__X__ NO

4. Are there documented human risks from exposure to the agent? (Risks may be determined from the MSDS or from references found in the Biosafety Manual.)

__X__ YES *(Complete Questions 4a-4e)* _____ NO *(Skip to Question 5)*

4a. Indicate the route(s) of human exposure:

__X__ Inhalation
__X__ Ingestion
_____ Other (describe below):
__X__ Contact
_____ Parenteral

4b. Describe the risks associated with the agent (mutagen, teratogen, etc.):

Can cause a rapid sudden increase in blood pressure; however, most overdoses require only patient observation because the material has a very short duration of action

4c. Indicate the acutely toxic/infectious dose in humans (in mg/kg or number of organisms):

LD50 in mice: 120 mg/kg; in rats 350 mg/kg
Only PI staff is exposed to the agent

4d. Describe any genetic changes to the organism and their suspected effects:

N/A

4e. Describe the symptoms of exposure: Exposure would have to be long term and contact would need to be ingested or contact with an open wound. Basic exposure symptoms would be irritation or skin rash.

4f. Describe the first aid methods to be taken in the case of exposure:

Flush the area with water

4g. Indicate all personal protection required:

X	Lab coat/dedicated clothing	____	Apron
X	Gloves	____	Face shield
X	Goggles	____	Respirator
X	Other (describe below): Mask		

5. Are there risks to other animals in the room or in the animal facility?

____ YES *(Complete Questions 5a-5d)* X NO *(Skip to Question 6)*

5a. Describe the risk to other animals:

5b. Indicate the route of animal exposure:

5c. describe all methods that will be used to contain the risk factor:

5d. Are special animal care requirements necessary?

____ YES *(Describe below)* ____ NO

6. Are special waste or carcass disposal requirements necessary?

____ YES *(Describe below)* X NO

7. Briefly describe the procedure(s) for administration of the agent and how the agent is metabolized and/or excreted. Include how long the agent is expected to remain in/be shed from the animal, the concentration of agent in any specific organs, etc.

Given iv over a 30 minute period; active agent likely catabolized by liver and excreted via kidney

7a. Please indicate whether or not the laboratory personnel and CompMed staff have been informed of these hazards and have received training on proper handling techniques.

	Laboratory Personnel		**CompMed Staff**
X	Yes	X	Yes
____	No	____	No

If not, please contact the Environmental Health and Safety Office to schedule the proper training.

8. Please provide any other relevant information regarding the agent (e.g., unique containment recommendations): This agent is prepared and infused iv by PI staff and is not handled by Comp Med staff.

1. Please indicate the type of hazardous agent being used. Check all that apply. For microorganisms, please list the Risk Group or Biosafety Level. For chemicals, please list the CAS # and the LD50 from the Material Safety Data Sheet (MSDS).

____ Radioactive material (Isotope: _____)
____ Infectious agent (Risk Group or Biosafety Level: _____)
____ Acutely toxic chemical (CAS #: _____, LD50: _____)
____ Known or suspected human carcinogen (Chemical Name: _____)
____ Known or suspected human mutagen/teratogen (Chemical Name: _____)
____ Recombinant DNA/RNA
____ Tissue sample (Type: _____)
__x__ Other (*Describe below*):

2. Please provide specific information about the agent:

Complete name
(Include strain for microorganisms): Sodium Nitroprusside
Dose and frequency of administration: 1 µg/kg BW/min/0.3ml saline for 10-15 min then 3 µg kg BW/0.3ml saline for 10-15 minutes
Concentration: stock solution 1 mg per ml prepared day of experiment:
Route: iv Duration of exposure: <60 minutes
How long will the animal be maintained after administration? >3 years
Animal species: Papio anubis Estimated animal weight: 4kg-18kg

3. Is the agent excreted or shed by the animal?

_____ YES *(Indicate the type of excreta and estimated quantity per day)*
__X__ NO

4. Are there documented human risks from exposure to the agent? (Risks may be determined from the MSDS or from references found in the Biosafety Manual.)

__X__ YES *(Complete Questions 4a-4e)* _____ NO *(Skip to Question 5)*

4a. Indicate the route(s) of human exposure:

__X__ Inhalation
__X__ Ingestion
_____ Other (describe below):
__X__ Contact
_____ Parenteral

4b. Describe the risks associated with the agent (mutagen, teratogen, etc.):

Agent is known to suppress blood pressure and produce bradycardia. This is unlikely as doses are relatively low.

4c. Indicate the acutely toxic/infectious dose in humans (in mg/kg or number of organisms):

Contact with acid liberates cyanide gas. May cause respiratory tract irritation. Hygroscopic and can cause eye and skin irritation. Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower lids until no evidence of chemical remains

4d. Describe any genetic changes to the organism and their suspected effects:

N/A

4e. Describe the symptoms of exposure: Formation/release of may result in headache, dizziness, weakness, collapse, and possible unconsciousness. Ingestion may result in symptoms similar to cyanide poisoning which is characterized by asphyxiation.

4f. Describe the first aid methods to be taken in the case of exposure:

Flush the area with water

4g. Indicate all personal protection required:

X	Lab coat/dedicated clothing	____	Apron
X	Gloves	____	Face shield
X	Goggles	____	Respirator
X	Other (describe below): Mask		

5. Are there risks to other animals in the room or in the animal facility?

____ YES *(Complete Questions 5a-5d)* X NO *(Skip to Question 6)*

5a. Describe the risk to other animals:

5b. Indicate the route of animal exposure:

5c. describe all methods that will be used to contain the risk factor:

5d. Are special animal care requirements necessary?

____ YES *(Describe below)* ____ NO

6. Are special waste or carcass disposal requirements necessary?

____ YES *(Describe below)* X NO

7. Briefly describe the procedure(s) for administration of the agent and how the agent is metabolized and/or excreted. Include how long the agent is expected to remain in/be shed from the animal, the concentration of agent in any specific organs, etc.

Given iv over a 30 minute period; active agent likely catabolized by liver and excreted via kidney

7a. Please indicate whether or not the laboratory personnel and CompMed staff have been informed of these hazards and have received training on proper handling techniques.

	Laboratory Personnel		**CompMed Staff**
X	Yes	X	Yes
____	No	____	No

If not, please contact the Environmental Health and Safety Office to schedule the proper training.

8. Please provide any other relevant information regarding the agent (e.g., unique containment recommendations): This agent is prepared and infused iv by PI staff and is not handled by Comp Med staff.

1. Please indicate the type of hazardous agent being used. Check all that apply. For microorganisms, please list the Risk Group or Biosafety Level. For chemicals, please list the CAS # and the LD50 from the Material Safety Data Sheet (MSDS).

   ____ Radioactive material (Isotope: _____)

   ____ Infectious agent (Risk Group or Biosafety Level: _____)

   ____ Acutely toxic chemical (CAS #: _____, LD50: _____)

   ____ Known or suspected human carcinogen (Chemical Name: _____)

   __X__ Known or suspected human mutagen/teratogen (Chemical Name: Estradiol 17B)

   ____ Recombinant DNA/RNA

   ____ Tissue sample (Type: _____)

   __x__ Other (*Describe below*):

2. Please provide specific information about the agent:

   Complete name
   (Include strain for microorganisms): Estradiol-17β 3-benzoate

   Dose and frequency of administration: 25 µg/kg BW daily for 35 days in pregnant baboons

   Concentration: 2mg/ml 115 µg/kg BW daily for 70 days in pregnant baboons

   Route: SC Duration of exposure: 35-70days (determined by study)

   How long will the animal be maintained after administration? >3 years

   Animal species: Papio anubis Estimated animal weight: 14-18kg

3. Is the agent excreted or shed by the animal?

   _____ YES *(Indicate the type of excreta and estimated quantity per day)*

   __X__ NO

4. Are there documented human risks from exposure to the agent? (Risks may be determined from the MSDS or from references found in the Biosafety Manual.)

   __X__ YES *(Complete Questions 4a-4e)* _____ NO *(Skip to Question 5)*

   4a. Indicate the route(s) of human exposure:

   __X__ Inhalation __X__ Contact

   __X__ Ingestion _____ Parenteral

   _____ Other (describe below):

   4b. Describe the risks associated with the agent (mutagen, teratogen, etc.):

   Cancers of the female reproductive tract are often due to exposure to endogenous estradiol and thus chronic exposure to estradiol 3 benzoate is likely also associated with increased risk for cancer.

   4c. Indicate the acutely toxic/infectious dose in humans (in mg/kg or number of organisms):

   Chronic estrogen exposure can induce menstrual irregularities and elicit estrogenic effects in males (e.g. breast enlargement). Estrogen is potent at low doses (µg/kg bw) but is rapidly (half-life of 90 minutes). Only PI staff is exposed to the agent

   4d. Describe any genetic changes to the organism and their suspected effects:

   N/A

4e. Describe the symptoms of exposure: Exposure would have to be long term and contact would need to be ingested or contact with an open wound. Basic exposure symptoms would be irritation or skin rash.

4f. Describe the first aid methods to be taken in the **case** of exposure:

Flush the area with water

4g. Indicate all personal protection required:

X	Lab coat/dedicated clothing	____	Apron
X	Gloves	____	Face shield
X	Goggles	____	Respirator
X	Other (describe below): Mask		

5. Are there risks to other animals in the room or in the animal facility?

____ YES *(Complete Questions 5a-5d)* X NO *(Skip to Question 6)*

5a. Describe the risk to other animals:

5b. Indicate the route of animal exposure:

5c. describe all methods that will be used to contain the risk factor:

5d. Are special animal care requirements necessary?

____ YES *(Describe below)* ____ NO

6. Are special waste or carcass disposal requirements necessary?

____ YES *(Describe below)* X NO

7. Briefly describe the procedure(s) for administration of the agent and how the agent is metabolized and/or excreted. Include how long the agent is expected to remain in/be shed from the animal, the concentration of agent in any specific organs, etc.

Estradiol benzoate is injected sc to the mother daily on days 25-59 of pregnancy and at 115 µg/kg/bw on days 100-175 days of gestation. The agent is first converted to the active hormone estradiol 17β (benzoate is removed) and then catabolized to estrone and glucuronylated and excreted via kidney/urine or via stool.

7a. Please indicate whether or not the laboratory personnel and CompMed staff have been informed of these hazards and have received training on proper handling techniques.

	Laboratory Personnel		**CompMed Staff**
X	Yes	X	Yes
____	No	____	No

If not, please contact the Environmental Health and Safety Office to schedule the proper training.

8. Please provide any other relevant information regarding the agent (e.g., unique containment recommendations): This agent is prepared and injected sc by PI staff and is not handled by Comp Med staff.

Eastern Virginia Medical School
Institutional Animal Care and Use Committee

JUN 25 2015

Attachment E: Animal Surgery

Project Title: Regulation of Fetal-Placental Development in the Primate

Protocol Number:

All animal use proposals involving surgery must provide specific details of pre-, intra-, and post-operative care and a plan for relief of pain and distress.

A. PRE-OPERATIVE PROCEDURE

1. List, by name and title, the person(s) responsible for evaluating the health status of the animals.

   Comp Med staff
   Gerald J. Pepe, Ph.D.
   [redacted] (Research Assoc II)
   [redacted] (Lab manager)

2. Will food be withheld?

   X YES (Below, please explain why food will be withheld and state how long it will be withheld.) ____ NO

   Animals are NPO overnight prior to sedation for surgery or other non-invasive procedure. This reduces vomiting and potential for aspiration during procedures.

3. List all **pre-operative anesthetic and/or analgesic agents** to be used (i.e., name and dosage for each agent).

   Initial sedation will be achieved using ketamine (10-15mg/kg) IM for all sedation.

4. Briefly describe how the animals will be prepared for surgery.

   On days ~60, ~100, ~170 of gestation based on the study group, baboons are sedated with ketamine (10-15 mg/kg), intubated and anesthetized with isoflurane/ oxygen and vitals (e.g. HR, BP, CO2, RR, and temperature) monitored by CompMed staff. A catheter is placed in the antecubital/brachial vein and IV fluids administered. A second catheter is placed in the saphenous vein for blood sampling using a 19g catheter 24inches in length and IV fluids administered (~1.6 ml/min over a 90 min period). The animal's abdomen/ surgical site is shaved and scrubbed with alcohol and Betadine solution. The animal is draped using sterile technique.

B. ANESTHETIC PROCEDURE

5. Will the animals be anesthetized?

   X YES (Complete Questions 6-8.) ____ NO (Below, please explain why the animals will not be anesthetized, then skip to Section C.)

6. List, by name and title, the person(s) who will administer the anesthesia.
A trained member of CompMed staff will be primarily responsible for anesthesia. When CompMed staff is not available, [redacted] will administer and monitor anesthesia.

7. List the anesthetic agent(s) to be used (i.e., name and dosage) and the route of administration for each agent. List, by name and title, the person(s) who will keep the anesthesia records.

Isoflurane gas vaporized with MAC of 1-3% in 100% oxygen via intubation tube. Anesthesia monitoring sheets are maintained by CompMed staff and stored in the animal record.

8. Explain how anesthetic recovery will be monitored and list, by name and title, the person(s) who will monitor the recovery.

At completion of the experiment, animal is given 1cc (10mg/kg) iron dextran IM. Catheters removed and animal will be monitored for swallowing reflex and response to stimuli before extubated. Vitals are monitored until extubated. Animals are then returned to their cages.

CompMed staff is primarily responsible for immediate anesthesia monitoring. [redacted] is also present.

C. POST-OPERATIVE PROCEDURE:

Investigators should refer to the IACUC *Post-Operative Care Guidelines* when developing a post-operative care plan.

***Appropriate post-operative records must be maintained in accordance with professionally accepted veterinary procedures regardless of the location of the animal. The attending veterinarian retains the authority to change post-operative care as necessary to ensure the comfort of the animal.* AWA, Section 13 and 9 CFR, April 14, 1997.**

9. List, by name and title, the person(s) who will monitor **daily post-operative care**.

Daily post-operative care is provided by [redacted], or if unavailable, CompMed staff in accordance to IACUC policy and consult with the attending veterinarian.

10. List, by name and title, the person(s) who will keep the post-operative records and list the location(s) where the records will be maintained.

Post-op monitoring sheet will be completed by [redacted] or, if unavailable, a member of CompMed staff. Monitoring sheets will become part of the animal medical record and kept in the animal facility.

11. Will post-operative analgesics be administered?

X YES _____ NO (Below, please explain why post-operative analgesia will not be used, then skip to Section D.)

12. Provide the following information for each **post-operative analgesic agent** to be administered:

Agent: Flunixin meglumine (Banamine)

Dose and Route: 2mg/kg IM Frequency: At surgery and 2days post operatively

Post-Operative Duration of Care: 5-7 days observation (BID for 3days SID for remainder)

Agent: Ketoprofen (as alternative to Banamine)

Dose and Route: 75mg / PO Frequency: IVGTT w/ biopsy and FMD w/ biopsy and 2days post operatively

Post-Operative Duration of Care: Monitoring for 5-7days

D. MULTIPLE SURVIVAL SURGERY

All major multiple survival surgery procedures must be conducted in accordance with the IACUC policy entitled, *Multiple Major Survival Surgery in Experimental Animals*.

The Animal Welfare Regulations state that an animal may not undergo more than one major survival surgery unless scientifically justified by the principal investigator, appropriate for the animal's veterinary care, or approved by the USDA Administrator. The IACUC must specifically review and approve every additional surgery to be performed on a single animal.

13. Will the animals be subjected to more than one survival surgery?

X YES (Complete Questions 13a-13b.) _____ NO (Skip to Question 14.)

13a. Please briefly outline the surgical procedures, explain how the surgeries are related, and **justify the need for more than one surgery per animal**.

The protocol is designed to elucidate the role of estrogen on placental fetal development and the function and impact on adrenocortical self-sufficiency in the perinatal period and metabolic and vascular function in adulthood. Thus surgeries are related to each other both by development and by estrogen. We study the animal at discrete times in control (no treatment), treated with Letrozole with or without estradiol 17β – 3 benzoate at early and late gestation. Thus each animal essentially serves as its own control. The major survival surgery performed is a cesarean section.

13b. How many surgeries will each animal undergo?

Each animal may undergo up to six (6) major survival surgeries without complications to the animal. While this is the optimal number to achieve statistically valid data, we work closely with the AV to ensure that animals are healthy and have no untoward medical and/or behavioral complications (excessive adhesions, uterine windows, endometriosis, etc.) that would not be compatible with performing further surgeries.

14. Have any animals to undergo major survival surgery in this protocol already undergone major survival surgery in any other IACUC protocol(s)?

X* YES (Complete Questions 14a-14d.) _____ NO (Skip to Section E.)

*However, they were not used in an unrelated protocol. This protocol is the 3-year continuation of ICUCU #12-010

14a. Identify all animals that have undergone prior surgical procedures in another protocol.

Please see surgery log submitted to the committee

14b. Identify all previous procedures performed on the animal(s) identified in Question 14a.

Please see surgery log submitted to the committee

14c. List the IACUC protocol number(s) under which the previous procedures were performed.

#12-010 (this submission is a 3-year renewal of 12-010)

14d. In accordance with the Animal Welfare Act, the IACUC must specifically review and approve all requests for the use of animals that have undergone previous survival surgery under another IACUC protocol. **Please justify the need to reuse such animals in this surgical protocol.**

The animals in this protocol were used in the prior approved IACUC protocol (research is continuous). The multiple use of the same baboon reduces the total number of animals required to conduct the study and still permit collection of statistically valid data. Thus we study the role of estrogen in the same baboon (i.e. experiments are interrelated/integrated) during control periods (e.g. on days 60 and ~170 of gestation). One animal rather than 5 animals are studied. Multiple pregnancies also mimics the situation in humans.

15. Surgery Classification for All Vertebrate Animal Species

SURGERY TYPE DEFINITIONS	Type 0 Surgery Surgical procedures performed with appropriate anesthesia that do not require the use of additional analgesia.	Type I Surgery Surgical procedures that result in mild pain and require pre-emptive use of at least one dose of additional analgesia pre- or perioperatively.	Type II Surgery Surgical procedures that result in moderate pain and require pre-emptive use of at least one dose of pre- or perioperative additional analgesia lasting at least 24-48 hours	Type III Surgery Surgical procedures or invasive manipulations that result in marked to severe pain and require pre-emptive use of at least one dose of pre- or peri-operative additional analgesia lasting at least 72 hours.
EXAMPLES OF PROCEDURES IN EACH SURGERY TYPE CLASSIFICATION	Small skin incision without deeper tissue manipulation; Skin biopsy; Placement of small subcutaneous implants; Tracheal injection; Retro-orbital bleed	Skin incision and deeper tissue manipulation, i.e., muscle incision, muscle biopsy, vessel cannulation (not percutaneous), vessel incision, vasectomy, non-extensive oral surgery and/or tissue manipulation, bone marrow aspiration	Laparotomy without organ manipulation or incision; Craniotomy (small burr hole); Placement of vascular access ports; Orchiectomy; Superficial lymph node biopsy; Thyroidectomy; Skin graft involving partial thickness and minimal body surface area	Laparotomy with significant organ manipulation or incision; Thoracotomy; Thymectomy; Orthopedic surgery to create or repair a bone fracture; Dissection into joints; Large craniotomy; Intraocular or corneal surgery/manipulation; Skin graft involving full thickness, multiple grafts, and/or extensive body surface area; Extensive oral or dental Surgery; Model involving extensive burns or tissue trauma; Parabiosis; Procedure involving infarction of organs with significant sensory innervation

Source Material: University of Michigan, University Committee on Use and Care of Animals, Policy on Analgesic Use in Animals Undergoing Surgery.

15a. Classify each surgical procedure to be performed according to the table listed above. **(Example: Surgery Type: 1; Procedure to be performed: Bone marrow aspiration.)**

Surgery Type: III Procedure to be performed: Cesarean section

Surgery Type: _____ Procedure to be performed: _____

Surgery Type: _____ Procedure to be performed: _____

E. SURGICAL PROCEDURES:

PLEASE SUBMIT A SEPARATE COPY OF THIS PAGE FOR EACH SURGICAL PROCEDURE TO BE PERFORMED.

PLEASE MAKE ADDITIONAL COPIES OF THIS PAGE AS NEEDED.

16. Is the procedure addressed on this page a major or a minor surgical procedure? **(The Animal Welfare Regulations define a major surgical procedure as one that penetrates and exposes a body cavity and/or a procedure that produces permanent impairment of physical or physiological functions. The *Guide* defines it as a procedure that penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection. A minor surgical procedure does not expose a body cavity and causes little or no physical impairment.)**

X Major surgical procedure _____ Minor surgical procedure

17. Is the procedure survival surgery?

X YES The answer is also No; please see non-survival for mother as stated in surgery procedure as outlined below in item #4, page 6 of Attachmnt E.)

18. Indicate the number of the surgical procedure described on this page (i.e., the only procedure to be performed, the 1st surgical procedure, the 2nd surgical procedure, etc.) If the same exact surgical procedure will be performed more than once on a single animal, indicate below how many times the procedure will be performed on the animal. In that case, submit only one copy of this page for the specific procedure to be performed.

The only major surgery performed in pregnant baboons is a cesarean section. C-sections are performed up to six times on one animal as long as no adverse health changes occur in the animal (inability to sustain pregnancy, repeated failure to become pregnant, unhealthy uterus) This would be determined in consult with the AV.

19. Indicate the location (*provide a specific building and room number if surgery will be conducted outside of the CompMed animal facility*), the time of day, and the day(s) of the week the surgery will be performed. Please take into consideration the time necessary for care following administration of anesthesia and the surgical procedure.

Surgical procedures take place on the [redacted] floor vivarium in surgery suite between 8am and 4pm, M-F

20. Describe the entire surgical procedure.

Once prepped as outlined in #4, the animal is draped using sterile technique. Blood samples (3-5ml) are obtained from the mother at '0' time, mid procedure and post placental delivery via maternal saphenous catheter. Blood chemistry will be evaluated using iStat analyzer. Prior to surgery, a proper plane of anesthesia will be assured by lack of response to a noxious stimulus such as abdominal pinch. A vertical mid-line incision is made using a 10 blade. The incision is 4-24cm in length depending on gestational age. Once the abdomen is open, the uterus is externalized and examined for placenta location. Mother's ovaries are examined for evidence of ovulation/corpus luteum. Two to four uterine vein blood samples are taken (5 ml) using a 23g needle. Bleeding is controlled by surgical gel foam. Warm sterile fluids are applied to the uterus as needed. Fetal crown is localized and a horizontal incision is made in the uterus to remove the fetus. A lap sponge can be used to absorb blood and reduce flow into the abdomen. A sample of amniotic fluid is recovered using a syringe. Once the fetus is carefully exteriorized, umbilical vein and artery samples are taken using a 23g butterfly set (4-8 ml) for hormone, metabolic and blood gas analyses. The umbilical cord is double clamped to ensure the safety of the mother. At this point, one of four procedures will follow: 1) fetus is euthanized and mother recovers; 2) fetus and mother are euthanized; 3) fetus and mother survive; 4) fetus survives and mother is euthanized (Non-survival). Details of each outcome are stated below.

1) Fetus is euthanized and the mother recovers: the fetus is then euthanized by injecting the umbilical artery with *B*euthanasia-D solution. After the fetus expires (no heartbeat) the cord is cut, placenta is manually delivered. Cardiac stick is used if second dose of euthanasia solution is needed after the cord has been cut. (this is rarely needed and the fetus is under initial effects of euthanasia solution). Fetal tissues are collected (e.g. liver, kidneys, lung, adipose adrenal, gonads, pancreas, skeletal muscle, heart, pituitary, aorta, carotid artery). Placenta is processed for analysis. The uterus is flushed with saline and sutured. Manual message is used to stimulate contractions. Once closed the uterus is placed back in the abdomen and the area flushed to remove any clots that may have accumulated. The abdomen is closed in three (3) layers when present (peritoneum, fascia and skin) using a combination of continuous and interrupted suture pattern. Absorbable suture is used, no need to remove suture at later date. Surgical glue can be used once skin is closed. Analgesia is given Flunixin meglumine (Banamine) with Iron Dextran IM. Anesthesia is stopped and the animal is monitored until swallowing or response to stimuli is present. Animal is extubated and catheters removed. Animal is returned to home cage and continued to be monitored until sitting upright. Immediate post-op is monitored by CompMed staff.

2) Fetus and mother are euthanized: the fetus is then euthanized by injecting the umbilical artery with *B*euthanasia-D solution. After the fetus expires (no heartbeat) the cord is cut, placenta is manually delivered. Cardiac stick is used if second dose of euthanasia solution is needed after the cord has been cut. (this is rarely needed and the fetus is under initial effects of euthanasia solution). Fetal tissues are collected (e.g. liver, kidneys, lung, adipose adrenal, gonads, pancreas, skeletal muscle, heart, pituitary, aorta, carotid artery). Placenta is processed for analysis. The mother is then euthanized by IV injection of *B*euthanasia-D solution and isoflurane gas elevated to highest level to ensure cessation. Upon confirmation of death, maternal tissue samples will be taken (liver, kidneys, lung, adrenal, gonads, pancreas, skeletal muscle, heart, adipose tissue)

3) Fetus and mother survive: after umbilical samples are taken, the cord is cut. The AV is ***always*** present for this procedure. Live neonates are cleared of mucous, stimulated to breathe, placed in warm blankets. The neonate will be reared by PI/CompMed staff under the guidance of the AV and SOP for rearing neonates. Once the cord is cut, Placenta is processed for analysis. The uterus is flushed with saline and sutured. Manual message is used to stimulate contractions. Once closed the uterus is placed back in the abdomen and the area flushed to remove any clots that may have accumulated. The abdomen is closed in three (3) layers when present (peritoneum, fascia and skin) using a combination of continuous and interrupted suture pattern. Absorbable suture is used, no need to remove suture at later date. Surgical glue can be used once skin is closed. Analgesia is given Flunixin meglumine (Banamine) with Iron Dextran IM. Anesthesia is stopped and the animal is monitored until swallowing or response to stimuli is present. Animal is extubated and catheters removed. Animal is returned to home cage and continued to be monitored until sitting upright. Immediate post-op is monitored by CompMed staff.

4) Fetus survives and mother is euthanized (Non-survival): after umbilical samples are taken, the cord is cut. The AV is ***always*** present for this procedure. Live neonates are cleared of mucous, stimulated to breathe, placed in warm blankets. The neonate will be reared by PI/CompMed staff under the guidance of the AV and SOP for rearing neonates. Placenta is processed for analysis. The mother is then euthanized by IV injection of *B*euthasia-D solution and isoflurane gas elevated to highest level to ensure cessation. Upon confirmation of death, maternal tissue samples will be taken (liver, kidneys, lung, adrenal, gonads, pancreas, skeletal muscle, heart, adipose tissue)

Eastern Virginia Medical School
Institutional Animal Care and Use Committee

Attachment E: Animal Surgery

Project Title: Regulation of Fetal-Placental Development in the Primate

Protocol Number: _____

All animal use proposals involving surgery must provide specific details of pre-, intra-, and post-operative care and a plan for relief of pain and distress.

A. PRE-OPERATIVE PROCEDURE

1. List, by name and title, the person(s) responsible for evaluating the health status of the animals.

   Comp Med staff
   Gerald J. Pepe, Ph.D.
   [redacted] (Research Assoc II)
   [redacted] (Lab manager)

2. Will food be withheld?

   X YES (Below, please explain why food will be withheld and state how long it will be withheld.) _____ NO

   Animals are NPO overnight prior to sedation for surgery or other non-invasive procedure. This reduces vomiting and potential for aspiration during procedures.

3. List all **pre-operative anesthetic and/or analgesic agents** to be used (i.e., name and dosage for each agent).

   Initial sedation will be achieved using ketamine (10-15mg/kg) IM for all sedation.

4. Briefly describe how the animals will be prepared for surgery.

   Briefly, baboons are fasted overnight, and sedated with ketamine. Animals are intubated and O2 is delivered. Back of both legs are shaved for catheter placement. Anesthesia is achieved via IV into an antecubital vein and a constant infusion of ketamine (0.1 mg/kg) and propofol (0.2 mg/kg) 0.6ml/minute. Animal is monitored for vitals (e.g. HR, BP, CO2, RR, and temperature) by CompMed staff. The animal's quadriceps are shaved to clear the area for biopsy and scrubbed with alcohol and Betadine solution. The area is draped using aseptic technique.

B. ANESTHETIC PROCEDURE

5. Will the animals be anesthetized?

   X YES (Complete Questions 6-8.) ___ NO (Below, please explain why the animals will not be anesthetized, then skip to Section C

6. List, by name and title, the person(s) who will administer the anesthesia.

   A trained member of CompMed staff will be primarily responsible for anesthesia. When CompMed staff is not available, [redacted] will administer and monitor anesthesia.

7. List the anesthetic agent(s) to be used (i.e., name and dosage) and the route of administration for each agent. List, by name and title, the person(s) who will keep the anesthesia records.

   Constant infusion of ketamine (0.1 mg/kg) and propofol (0.2 mg/kg) 0.6ml/minute. Records are kept by comp med

8. Explain how anesthetic recovery will be monitored and list, by name and title, the person(s) who will monitor the recovery.

   At completion of the experiment, catheters removed and animal will be monitored for swallowing reflex and response to stimuli before extubated. Vitals are monitored until extubated. Animals are then returned to their cages.

   CompMed staff is primarily responsible for immediate anesthesia monitoring. [redacted] is also present.

C. POST-OPERATIVE PROCEDURE:

Investigators should refer to the IACUC *Post-Operative Care Guidelines* when developing a post-operative care plan.

***Appropriate post-operative records must be maintained in accordance with professionally accepted veterinary procedures regardless of the location of the animal. The attending veterinarian retains the authority to change post-operative care as necessary to ensure the comfort of the animal.* AWA, Section 13 and 9 CFR, April 14, 1997.**

9. List, by name and title, the person(s) who will monitor **daily post-operative care**.

   Daily post-operative care is provided by [redacted], or if unavailable, CompMed staff in accordance to IACUC policy and consult with AV.

10. List, by name and title, the person(s) who will keep the post-operative records and list the location(s) where the records will be maintained.

    Post-op monitoring sheet will be completed by [redacted] or, if unavailable, a member of CompMed staff. Monitoring sheets will become part of the animal medical record and kept in the animal facility.

11. Will post-operative analgesics be administered?

    X YES ___ NO (Below, please explain why post-operative analgesia will not be used, then skip to Section D.)

12. Provide the following information for each **post-operative analgesic agent** to be administered:

    Agent: Flunixin meglumine (Banamine)

    Dose and Route: 2mg/kg IM Frequency: IVGTT w/ biopsy and FMD w/ biopsy and 2days post operatively

    Post-Operative Duration of Care: 5-7 days observation (BID for 3days SID for remainder)

    Agent: Ketoprofen (as alternative to Banamine)

    Dose and Route: 75mg / PO Frequency: IVGTT w/ biopsy and FMD w/ biopsy and 2days post operatively

    Post-Operative Duration of Care: Monitoring for 5-7days

D. MULTIPLE SURVIVAL SURGERY

All major multiple survival surgery procedures must be conducted in accordance with the IACUC policy entitled, *Multiple Major Survival Surgery in Experimental Animals*.

The Animal Welfare Regulations state that an animal may not undergo more than one major survival surgery unless scientifically justified by the principal investigator, appropriate for the animal's veterinary care, or approved by the USDA Administrator. The IACUC must specifically review and approve every additional surgery to be performed on a single animal.

13. Will the animals be subjected to more than one survival surgery?

X YES (Complete Questions 13a-13b.) _____ NO (Skip to Question 14.)

13a. Please briefly outline the surgical procedures, explain how the surgeries are related, and **justify the need for more than one surgery per animal.**

The protocol is designed to evaluate change in response over time and the function and impact on metabolic and vascular function over adolescent growth through puberty and into adulthood.

13b. How many surgeries will each animal undergo?

Each animal may undergo up to six (3) muscle biopsy procedure over a course of 15years, one at (~24-30 months and the 2nd and 3rd at one-two year intervals after puberty into adulthood to age 15 years). While this is the optimal number to achieve statistically valid data, we work closely with the AV to ensure that animals are healthy and have no untoward medical and/or behavioral complications (reduction in movement, adverse change in behavior) that would not be compatible with performing further surgeries.

14. Have any animals to undergo major survival surgery in this protocol already undergone major survival surgery in any other IACUC protocol(s)?

_____ YES (Complete Questions 14a-14d.) X NO (Skip to Section E.)

14a. Identify all animals that have undergone prior surgical procedures in another protocol.

14b. Identify all previous procedures performed on the animal(s) identified in Question 14a.

14c. List the IACUC protocol number(s) under which the previous procedures were performed.

14d. In accordance with the Animal Welfare Act, the IACUC must specifically review and approve all requests for the use of animals that have undergone previous survival surgery under another IACUC protocol. **Please justify the need to reuse such animals in this surgical protocol.**

The animals in this protocol were used in the prior approved IACUC protocol (research is continuous). The multiple use of the same baboon reduces the total number of animals required to conduct the study and still permit collection of statistically valid data. These animals are exposed to minor surgical manipulation.

15. Surgery Classification for All Vertebrate Animal Species

SURGERY TYPE DEFINITIONS	**Type 0 Surgery** Surgical procedures performed with appropriate anesthesia that do not require the use of additional analgesia.	**Type I Surgery** Surgical procedures that result in mild pain and require pre-emptive use of at least one dose of additional analgesia pre- or perioperatively.	**Type II Surgery** Surgical procedures that result in moderate pain and require pre-emptive use of at least one dose of pre- or perioperative additional analgesia lasting at least 24-48 hours.	**Type III Surgery** Surgical procedures or invasive manipulations that result in marked to severe pain and require pre-emptive use of at least one dose of pre- or perioperative additional analgesia lasting at least 72 hours.
EXAMPLES OF PROCEDURES IN EACH SURGERY TYPE CLASSIFICATION	Small skin incision without deeper tissue manipulation; Skin biopsy; Placement of small subcutaneous implants; Tracheal injection; Retro-orbital bleed	Skin incision and deeper tissue manipulation, i.e., muscle incision, muscle biopsy, vessel cannulation (not percutaneous), vessel incision, vasectomy, non-extensive oral surgery and/or tissue manipulation, bone marrow aspiration	Laparotomy without organ manipulation or incision; Craniotomy (small burr hole); Placement of vascular access ports; Orchiectomy; Superficial lymph node biopsy; Thyroidectomy; Skin graft involving partial thickness and minimal body surface area	Laparotomy with significant organ manipulation or incision; Thoracotomy; Thymectomy; Orthopedic surgery to create or repair a bone fracture; Dissection into joints; Large craniotomy; Intraocular or corneal surgery/manipulation; Skin graft involving full thickness, multiple grafts, and/or extensive body surface area; Extensive oral or dental Surgery; Model involving extensive burns or tissue trauma; Parabiosis; Procedure involving infarction of organs with significant sensory innervation

Source Material: University of Michigan, University Committee on Use and Care of Animals, Policy on Analgesic Use in Animals Undergoing Surgery.

15a. Classify each surgical procedure to be performed according to the table listed above. **(Example: Surgery Type: 1; Procedure to be performed: Bone marrow aspiration.)**

Surgery Type: I Procedure to be performed: Muscle biopsy

Surgery Type: 0 Procedure to be performed: Amniocentesis at IVGTT

Surgery Type: _____ Procedure to be performed: _____

E. SURGICAL PROCEDURES:

PLEASE SUBMIT A SEPARATE COPY OF THIS PAGE FOR EACH SURGICAL PROCEDURE TO BE PERFORMED.

PLEASE MAKE ADDITIONAL COPIES OF THIS PAGE AS NEEDED.

16. Is the procedure addressed on this page a major or a minor surgical procedure? **(The Animal Welfare Regulations define a major surgical procedure as one that penetrates and exposes a body cavity and/or a procedure that produces permanent impairment of physical or physiological functions. The *Guide* defines it as a procedure that penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection. A minor surgical procedure does not expose a body cavity and causes little or no physical impairment.)**

_____ Major surgical procedure X Minor surgical procedure

17. Is the procedure survival surgery?

X YES _____ NO

18. Indicate the number of the surgical procedure described on this page (i.e., the only procedure to be performed, the 1st surgical procedure, the 2nd surgical procedure, etc.) If the same exact surgical procedure will be performed more than once on a single animal, indicate below how many times the procedure will be performed on the animal. In that case, submit only one copy of this page for the specific procedure to be performed.

1st surgical procedure: Muscle biopsy at IVGTT and/or FMD with infusion of pharmaceutical agent. Each animal may undergo up to six (3) muscle biopsy procedure over a course of 15years. (~24-30 months and at one-two year intervals after puberty into adulthood to age 15 years)

19. Indicate the location (*provide a specific building and room number if surgery will be conducted outside of the CompMed animal facility*), the time of day, and the day(s) of the week the surgery will be performed. Please take into consideration the time necessary for care following administration of anesthesia and the surgical procedure.

Surgical procedures take place on the [redacted] floor vivarium in surgery suite between 8am and 4pm, M-F

20. Describe the entire surgical procedure.

Briefly, baboons are fasted overnight, and sedated with ketamine. Animals are intubated and O_2 is delivered. Anesthesia is achieved via IV into an antecubital vein and a constant infusion of ketamine (0.1 mg/kg) and propofol (0.2 mg/kg) 0.6ml/minute. Once sedated, two base line blood samples are collected and blood glucose and blood chemistries/gas levels determined using IStat. A small incision (0.5cm – 1cm) is made exposing the vastus lateralis. At time '0', a 2.5-3.0cm(L) x 0.5-0.75cm(W) x 0.2-0.25(D) muscle sample is surgically removed using a 15 scalpel blade from alternating legs. The area is packed with gel foam to minimize bleeding. Sample taken at ~24-30 months, a small single knot is placed in the fascia using non-absorbable suture. This is to act as a reference point for future experiments as the animal grows. The fascia and skin are then closed with absorbable suture.

At experimental time zero, a bolus of glucose (0.25 grams/kg BW) is injected over a 10-15 second period into the antecubital vein. Blood samples (total blood sample volume < 10% total blood volume/not to exceed 10 ml/kg/month) are collected from the saphenous vein catheter at 1, 3, 5, 10, 20, and 30 mins. At time '30', a second biopsy is taken from the alternate leg following the same procedure. During the experiment, the animal is constantly monitored for BP, HR, respiration, and warmed via warming blanket. Samples are taken to measure rise in insulin levels and allow us to determine responsivity of skeletal muscle (e.g. expression of insulin signaling molecules; metabolic enzymes as determined by Western blot/RT-PCR) and relate findings to insulin sensitivity/resistance as determined by the ivGTT. At completion of the experiment, animal is given 1cc (10mg/kg) iron dextran IM. Catheters removed and animal will be monitored for swallowing reflex and response to stimuli before extubated. Animals are then returned to their cages

For muscle biopsy at FMD with infusion of pharmaceutical agent, the procedure is the same pre and post infusion of the agent.

E. SURGICAL PROCEDURES:

PLEASE SUBMIT A SEPARATE COPY OF THIS PAGE FOR EACH SURGICAL PROCEDURE TO BE PERFORMED.

PLEASE MAKE ADDITIONAL COPIES OF THIS PAGE AS NEEDED.

16. Is the procedure addressed on this page a major or a minor surgical procedure? **(The Animal Welfare Regulations define a major surgical procedure as one that penetrates and exposes a body cavity and/or a procedure that produces permanent impairment of physical or physiological functions. The *Guide* defines it as a procedure that penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection. A minor surgical procedure does not expose a body cavity and causes little or no physical impairment.)**

_____ Major surgical procedure X Minor surgical procedure

17. Is the procedure survival surgery?

X YES _____ NO

18. Indicate the number of the surgical procedure described on this page (i.e., the only procedure to be performed, the 1st surgical procedure, the 2nd surgical procedure, etc.) If the same exact surgical procedure will be performed more than once on a single animal, indicate below how many times the procedure will be performed on the animal. In that case, submit only one copy of this page for the specific procedure to be performed.

2nd surgical procedure: Amniocentesis during IVGTT of pregnant baboon ~80-120d gestation.

19. Indicate the location (*provide a specific building and room number if surgery will be conducted outside of the CompMed animal facility*), the time of day, and the day(s) of the week the surgery will be performed. Please take into consideration the time necessary for care following administration of anesthesia and the surgical procedure.

Surgical procedures take place on the [redacted] floor vivarium in surgery suite between 8am and 4pm, M-F

20. Describe the entire surgical procedure.

At completion of IVGTT in adult pregnant baboons as described previously, a 10ml sample of amniotic fluid will be obtained for sex determination. Briefly, under Ketafol, fetal/placental position is determined using GE Logic+ ultrasound. The area is cleaned with alcohol and an 18g x 2in needle inserted through the uterine wall into the amniotic cavity and 10 ml collected. Fetal HR is rechecked. This will take place at the completion of the IVGTT to ensure complete sedation of the animal. At completion of the experiment, animal is given 1cc (10mg/kg) iron dextran IM. Catheters removed and animal will be monitored for swallowing reflex and response to stimuli before extubated. Animals are then returned to their cages

IACUC Surgery Documentation for Multiple Survival Surgeries

boon #	sx date	sx date	sx date	sx date	sx date	sx date	sx
18631	Jul-09	Oct-10	Feb-15				3
No noted surgical complications							
18660	Jan-10	Sep-10	Feb-12				3
No noted surgical complications							
18652	Mar-11	Apr-12					2
No noted surgical complications							
19045	8/09[bc]	11/10[bc]					2
No noted surgical complications							
28768	Nov-14						1
No noted surgical complications							
26745	Dec-14						1
No noted surgical complications							

EVMS born animals

boon #	sx date	sx date	sx date	sx date	sx date	sx date
11790A	Jan-08					
G194	Dec-08					
H080	Oct-09					
I096	Dec-04					

All surgeries were ovariectomies and were conducted with no complications.

EVMS
Eastern Virginia Medical School

August 10, 2015

Gerald J. Pepe, Ph.D.
Chair, Department of Physiological Sciences
Eastern Virginia Medical School
[redacted]
Norfolk, Virginia 23507

Dear Dr. Pepe:

The Institutional Animal Care and Use Committee has reviewed your amendment request (*i.e., intramuscular injection of 17β-Estradiol and 17β-Estradiol-3-benzoate into a near-term fetus (Day 160-175 of gestation) under Isoflurane anesthesia and ultrasound to demonstrate that the agent injected directly into the fetus can elicit a relatively rapid effect on signaling mechanisms and production of proteins such as VEGF; the fetus will be delivered by cesarean section 6 or 24 hours later as outlined in the approved protocol*) to the protocol entitled, ***Regulation of Fetal-placental Development in the Primate*** **(IACUC #15-009). The amendment to the protocol has been approved via the facilitated review (FR) process.**

Although the Division of Comparative Medicine (CompMed) will make every effort to provide housing, husbandry, and veterinary support for your research project, IACUC approval of this amendment request does not guarantee that CompMed will be able to accommodate the work immediately. Available space and resources within the CompMed animal facility are determined by the number of ongoing projects and the animal census at any given time. Please consult with the CompMed management team to confirm availability prior to implementing the approved modification(s).

It is the responsibility of the principal investigator to notify the Committee, in writing, of any deviation from the IACUC-approved protocol. Please note that a change in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian prior to submitting the amendment request to the IACUC for approval.

It is also the responsibility of the principal investigator to provide for regular observation of the health of the research animals and to provide the Division of Comparative Medicine (CompMed) with the name(s) and telephone number(s) of the person(s) who may be contacted and/or take action in the event that the CompMed staff notes an emergency condition with the research animals.

Thank you for your continued cooperation with the Institutional Animal Care and Use Committee.

Sincerely,

[redacted]
Institutional Animal Care and Use Committee

Office of Research
[redacted]
NORFOLK, VA 23507
m: 757[redacted]
fx: 757[redacted]
www.evms.edu

[redacted]

cc: [redacted]
Attending Veterinarian
Division of Comparative Medicine

[redacted]
Project Manager
Division of Comparative Medicine

[redacted]
Senior Associate Dean for Research
Institutional Official

EVMS
Eastern Virginia Medical School

August 3, 2015

Gerald J. Pepe, Ph.D.
Professor and Chair

[redacted] Chair
Institutional Animal Care and Use Committee
Eastern Virginia Medical School
Norfolk, VA 23507

AUG 4 2015

Dear [redacted]:

I would like to have the appended research protocol (briefly outlined below) amended to my approved IACUC protocol entitled "Regulation of Fetal-Placental Development in the Primate" (#15-009). In the existing protocol, we are approved to treat pregnant baboons with letrozole between days 100 and 175 of gestation to block estrogen production and determine impact on fetal development including ontogeny of vascular endothelial cell growth factor (VEGF) by endothelial cells and insulin signalling molecules in skeletal muscle obtained from fetuses delivered at day 175 of gestation by cesarean section. Another groups of baboons is treated with letrozole and estradiol chronically (days 100-175) to restore estrogen levels. However, while this chronic estrogen paradigm is important, it is imperative that we demonstrate that estrogen injected directly into the fetus can elicit a relatively rapid (6-24 hours) effect on signalling mechanisms and production of proteins such as VEGF. Accordingly, we are requesting that we be approved to inject the fetus im under ultrasound and ketamine-isoflurane (nose-cone) anesthesia with 1.0 ml saline containing 2 µg estradiol and 2 µg estradiol benzoate (will allow estradiol to be in circulation longer) in 0.05 ml (50 µl) ethanol and deliver the fetus 6 hours or 24 hours later as already approved in our protocol. Basically, on day 175 of gestation, maternal baboons fasted overnight will be sedated with ketamine (10 mg/kg bw) and lightly anesthetized with isoflurane via mask and the amniotic fluid/placenta/fetus identified by ultrasound. The fetus at this stage is relatively large (750-900 grams), easy to visualize, almost completely fills the uterine cavity and can be moved up against the uterine wall. Once localized, a 3 inch 23 gauge needle is inserted through the uterine wall into the fetal rump or shoulder and estradiol in 1.0 ml saline/5% ethanol injected. The needle is removed and fetal heart rate checked to assure stability. Baboon mothers are then returned to their cage and prepared for surgery and cesarean section 6 or 24 hours later as outlined in our approved protocol. We have performed similar studies in the past and published manuscripts describing the procedure in which near term (day 165-175) as well as midgestation (day 100) baboon fetuses were injected im under ultrasound with 0.5 ml solution of betamethasone/Celestone-Soluspan (Pepe et al, 1996 Endocrinology 137:3323; paper appended; Aberdeen et al, 1998; JCEM, 83:976). The IACUC's time and effort in reviewing this amendment are most appreciated.

Sincerely,

Gerald J. Pepe, Ph.D.
Professor and Chairman

Physiological Sciences
[redacted]
P.O. BOX 1980
NORFOLK, VA 23501-1980
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f: 757[redacted]
www.evms.edu

April 2010
Updated February 2011; March 2011; April 2012; August 2013

REQUEST TO AMEND A PREVIOUSLY APPROVED IACUC PROTOCOL

Institutional Animal Care and Use Committee (IACUC)

Eastern Virginia Medical School
Office of Research

IACUC Office
[redacted]

Office: 757-[redacted]
Fax: 757-[redacted]

RECEIVED
AUG 4 2015
OFFICE OF RESEARCH

FOR OFFICE USE ONLY
Date Received: 7/4/15 [redacted] **Review Method:** ___ FCR / X FR / ___ Administrative (*Personnel Changes Only*)
IBC Approval? X Yes / ___ No **IBC Approval Date:** 7/4/15 **Final Approval Date:** 7/6/15

General Information and Instructions:

1. All requested amendments must be approved by the IACUC **before** they are implemented.
2. Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian **before** the changes are submitted to the IACUC Office.
3. A request to increase the number of animals assigned to a protocol must be approved by the IACUC **before** the animals are ordered.
4. **Submit the original signed amendment form to the IACUC Office located in [redacted], and e-mail the MSWord version of the form to the IACUC Administrator.**
5. ***The IACUC reserves the right to require submission of an IACUC "Initial Review Form" based upon the Committee's assessment of the amendment(s) requested herein.***

I. ADMINISTRATIVE INFORMATION

Protocol Number: 15-009	**Protocol Initial Approval Date: 07-2015** 7/9/15 [redacted]
Protocol Title: Regulation of Fetal-placental Development in the Primate	
Principal Investigator: Gerald J Pepe	
Approved USDA Pain Code Level(s) – *not required for personnel additions only*: **B,C & D**	

II. PERSONNEL

List all personnel to be added to or removed from the protocol. ***All persons authorized to work on the protocol must have completed the required CITI/LATA and OHSP training modules and must have completed a health/risk assessment with the Occupational Health Department.***

Name	Added (+) or Removed (-)	E-mail Address *(additions only)*	CITI/LATA Training Certification Number *(additions only)*	CITI/LATA Training Species Module(s) Completed *(additions only)*	OHSP Training Certification Number *(additions only)*	Occupational Health/ Risk Assessment Date *(additions only)*
1.						
2.						
3.						
Procedure(s) to be performed by personnel addition #1:						
Procedure(s) to be performed by personnel addition #2:						
Procedure(s) to be performed by personnel addition #3:						

III. PROPOSED CHANGES TO THE ORIGINALLY APPROVED PROTOCOL

☐ **A. CHANGE OF SPONSOR/FUNDING SOURCE**

List the new/approved funding source, the project/grant number, the project/grant title (if different from the IACUC protocol title), and the award period.

☐ **B. CHANGE IN PROJECT SITE(S)**

List all new locations (i.e., building and room number(s)) and indicate what procedure(s) will be performed in each new location.

☐ **C. CHANGE OF SPECIES AND/OR STRAIN**

List the species to be added to or removed from the protocol. Please provide the rationale for any species/strain additions and indicate the USDA Pain Category assignment for each new species/strain. Also, list any anticipated changes in veterinary care and/or husbandry due to the new species/strain.

Species and/or strain	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E

☐ **D. CHANGE IN ANIMAL NUMBERS**

List the number of animals to be added to or removed from the protocol. If animals will be added, please provide the justification for the increase and indicate the number of animals to be assigned to each USDA Pain Category.

Species and/or strain	Year (1, 2 or 3)	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E	TOTAL

Will the animals be ordered through CompMed? _____Yes _____ No ***(Identify the source and provide the rationale/justification below.)***

Will the animals require special housing (e.g., specific bedding requirements, isolator cages, special feed, etc.)
_____ Yes ***(Please specify below.)*** _____ No

USDA Pain Code B *= Breeding or holding colony; no animal manipulation.*
USDA Pain Code C *= Procedures involving no or momentary slight pain or distress for which no pain-relieving drugs are used.*
USDA Pain Code D *= Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.*
USDA Pain Code E *= Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.*

[X] **E. CHANGE IN EXPERIMENTAL PROCEDURES AND/OR INSTRUMENTATION**

Describe, in detail, the proposed change(s). *Please indicate potentially painful/distressing effects on the animals as a result of the proposed change(s). Also, please indicate any expected adverse effects and any changes in monitoring, husbandry, housing, early endpoint, and/or experimental endpoint. Finally, please complete the checklist below.*

We are request to inject the fetus im under ultrasound and isoflurane/O2 (nose-cone) anesthesia with 1.0 ml saline containing 2 µg estradiol and 2 µg estradiol benzoate (will allow estradiol to be in circulation longer) in 0.05 ml (50 µl) ethanol. We would then deliver the fetus 6 hours or 24 hours later as already approved in our protocol. Basically, on day 160-175 of gestation, maternal baboons fasted overnight will be sedated with ketamine (10 mg/kg bw) and lightly anesthetized with isoflurane via mask and the amniotic fluid/placenta/fetus identified by ultrasound. The fetus at this stage is relatively large (750-900 grams), easy to visualize, almost completely fills the uterine cavity and can be moved up against the uterine wall. Once localized, a 3 inch 23 gauge needle is inserted through the uterine wall into the fetal rump or shoulder and estradiol in 1.0 ml saline/5% ethanol injected. The needle is removed and fetal heart rate checked to assure stability. Baboon mothers are then returned to their cage and prepared for surgery and cesarean section 6 or 24 hours later as outlined in our approved protocol. We have performed similar studies in the past and published manuscripts describing the procedure in which near term (day 165-175) as well as mid gestation (day 100) baboon fetuses were injected im under ultrasound with 0.5 ml solution of betamethasone/Celestone-Soluspan (Pepe et al, 1996 Endocrinology 137:3323; Aberdeen et al, 1998; JCEM, 83:976).

The procedure does not require analgesia as it mimics the approved amniocentesis procedure. Any distress to the fetus can be monitored via ultrasound following injection. Delivery of the fetus would be no later than 24 hours post injection. No adverse effects should arise to the mother. Any observed abnormal bleeding or changes in vital will be noted and the AV contracted.

There is no change to anesthesia.

Please check all that apply to the proposed change(s). Complete the required IACUC Attachment and submit it along with the amendment form.	
Biohazardous Agents: ____ Yes __x__ No (e.g., recombinant DNA, RNA, all tissue or cell samples, laboratory-induced infection, cultured pathogens)	Complete and submit **Attachment D**
Radioisotopes, *in vivo*: ____ Yes ___x_ No	Complete and submit **Attachment D**

***USDA Pain Code B** = Breeding or holding colony; no animal manipulation.*
***USDA Pain Code C** = Procedures involving no or momentary slight pain or distress for which no pain-relieving drugs are used.*
***USDA Pain Code D** = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.*
***USDA Pain Code E** = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.*

Chemical Agents: __x__ Yes ____ No (e.g., known or suspected chemical hazards, mutagens, or teratogens)	Complete and submit **Attachment D**
Stress or Prolonged Restraint: ____ Yes __x__ No	Complete and submit **Attachment C**
Food and/or Water Deprivation: ____ Yes _x___ No	Complete and submit **Attachment C**
Surgical Procedures: ____ Yes __x__ No (i.e., single survival, multiple survival, or non-survival)	Complete and submit **Attachment E**
Antibody Production: ____ Yes __x__ No	Complete and submit **Attachment F**
Toxicity Testing (LD50): ____ Yes _x___ No	Complete and submit **Attachment G**
Lasers or Penetrating Electromagnetic Radiation: ____ Yes __x__ No	
Collection of Tissues, Cells, or Organs: ____ Yes __x__ No (Please explain below.)	
Tumor Transplantation or Induction: ____ Yes ___x_ No	
Special Diet: ____ Yes __x__ No (Please explain below.)	
Other : N/A (Please specify below.)	

X **F. CHANGE IN ANESTHESIA, THERAPEUTICS, AND/OR EXPERIMENTAL AGENTS**

	Dose (mg/kg)	Route of Administration	Frequency of Administration	
Sedatives/Tranquilizers				
Anesthetics - General				
Anesthetics - Local				
				Length of Administration
Analgesics				
Antibiotics				

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

Miscellaneous				

EXPERIMENTAL AGENTS:

Agent: 17β-Estradiol and 17β-Estradiol-3-benzoate **Agent Vehicle:** 1ml saline + 0.05ml ethanol

Route/Site: IM to fetus **Volume per administration:** ~1.05ml

Frequency of administration: Once at (near) term; 6-24 hours before cesarean delivery

Expected side effects and/or changes in animal behavior:

No expected change in behavior. Side effect could be distress to the fetus

Agent: _____ **Agent Vehicle:** _____

Route/Site: _____ **Volume per administration:** _____

Frequency of administration: _____

Expected side effects and/or changes in animal behavior: _____

Agent: _____ **Agent Vehicle:** _____

Route/Site: _____ **Volume per administration:** _____

Frequency of administration: _____

Expected side effects and/or changes in animal behavior: _____

INVESTIGATOR ASSURANCES:

X	*I understand that it is my responsibility as the Principal Investigator to ensure that all personnel who work on this protocol are adequately trained. That training may be provided by me, an experienced member of the investigative staff, the Attending Veterinarian, a qualified Division of Comparative Medicine (CompMed) staff member, or an appropriate outside source. I hereby affirm that each person added to this protocol by way of this amendment request has been adequately trained to perform the procedure(s) indicated above. Any person who has not been adequately trained will be so trained prior to performing the procedure(s).*
X	*No animal involved in this project will be subjected to discomfort, pain, or distress, unless it is unavoidable in the conduct of scientifically valuable research and approval of such has been approved by the IACUC.*
X	*I agree to comply with all federal and institutional policies governing the use of animals used in this project.*

***USDA Pain Code B** – Breeding or holding colony; no animal manipulation.*
***USDA Pain Code C** – Procedures involving no or momentary slight pain or distress for which no pain-relieving drugs are used.*
***USDA Pain Code D** – Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.*
***USDA Pain Code E** – Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.*

PRINCIPAL INVESTIGATOR:	
Printed Name: Gerald J. Pepe, Ph.D.	
Signature:	**Date:** 8/3/2015

APPROVAL SIGNATURES:	
VETERINARY PRE-REVIEW: *Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian before the changes are submitted to the IACUC for review.* **Attending Veterinarian Printed Name:** **Attending Veterinarian Signature:**	**Date:**
FINAL IACUC APPROVAL: *All revisions must be approved by the IACUC prior to implementation.* **IACUC Chair or Vice Chair Printed Name:** [redacted] **IACUC Chair or Vice Chair Signature:** [redacted]	August 12, 2015 **Date:**

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

Eastern Virginia Medical School
Institutional Animal Care and Use Committee

AUG 4 2015

Attachment D: Use of Hazardous Agents

Project Title: Regulation of fetal placental development in the primate

This form applies to IACUC protocols using materials such as radioisotopes, infectious agents, carcinogens, biohazards, mutagens, teratogens, abortifacents, acutely toxic chemicals, or other potentially hazardous chemicals. Information provided in this form will enable assessment of risk to individuals handling or caring for animals, as well as risk to other animals. **If you need assistance answering any of the questions, please contact the Environmental Health and Safety Office at (757) 446-5798.**

The Division of Comparative Medicine (CompMed) must be notified in writing at least one week prior to the use of hazardous agents in animals. The use of hazardous agents in animals is not permitted until approval has been granted by the CompMed Facility Manager. Please reference the CompMed SOP entitled, *Appropriate Notification, Signage and Protocol for Working with Hazardous Agents in Animal Holding Rooms and Procedure Rooms.*

YOU MUST COMPLETE A SEPARATE FORM FOR EACH HAZARDOUS AGENT USED.

1. Please indicate the type of hazardous agent being used. Check all that apply. For microorganisms, please list the Risk Group or Biosafety Level. For chemicals, please list the CAS # and the LD50 from the Material Safety Data Sheet (MSDS).

    ____ Radioactive material (Isotope: _____)
    ____ Infectious agent (Risk Group or Biosafety Level: _____)
    ____ Acutely toxic chemical (CAS #: _____, LD50: _____)
    ____ Known or suspected human carcinogen (Chemical Name: _____)
    __X__ Known or suspected human mutagen/teratogen (Chemical Name: _____)
    ____ Recombinant DNA/RNA
    ____ Tissue sample (Type: _____)
    ____ Other (*Describe below*):

2. Please provide specific information about the agent:

Complete name
(Include strain for microorganisms): β-estradiol

Dose and frequency of administration: 1ml in saline / 5% ethanol X1

Concentration: 2 µg

Route: IM Duration of exposure: 6-24 hours

How long will the animal be maintained after administration? 6-24 hours

Animal species: NHP Estimated animal weight: 700-900 grams

3. Is the agent excreted or shed by the animal?

____ YES *(Indicate the type of excreta and estimated quantity per day)*

X NO

4. Are there documented human risks from exposure to the agent? (Risks may be determined from the MSDS or from references found in the Biosafety Manual.)

X YES *(Complete Questions 4a-4e)* ____ NO *(Skip to Question 5)*

4a. Indicate the route(s) of human exposure:

X Inhalation
X Ingestion
____ Other (describe below):
X Contact
____ Parenteral

4b. Describe the risks associated with the agent (mutagen, teratogen, etc.):

Cancers of the female reproductive tract are often due to exposure to endogenous estradiol and thus chronic exposure is likely also associated with increased risk of cancer.

4c. Indicate the acutely toxic/infectious dose in humans (in mg/kg or number of organisms):

Chronic exposure can induce menstrual irregularities and elicit estrogenic effects in males. Only PI staff is exposed to the agent in raw form.

4d. Describe any genetic changes to the organism and their suspected effects:

N/A

4e. Describe the symptoms of exposure:

Exposure would have to be long term.

4f. Describe the first aid methods to be taken in the case of exposure:

Flush the area with water and contact Occ Health

4g. Indicate all personal protection required:

x	Lab coat/dedicated clothing		Apron
x	Gloves		Face shield
	Goggles		Respirator
X	Other (describe below):		

Face mask

5. Are there risks to other animals in the room or in the animal facility?

	YES *(Complete Questions 5a-5d)*	x	NO *(Skip to Question 6)*

5a. Describe the risk to other animals:

5b. Indicate the route of animal exposure:

5c. Describe all methods that will be used to contain the risk factor:

5d. Are special animal care requirements necessary?

	YES *(Describe below)*		NO

6. Are special waste or carcass disposal requirements necessary?

	YES *(Describe below)*	x	NO

7. Briefly describe the procedure(s) for administration of the agent and how the agent is metabolized and/or excreted. Include how long the agent is expected to remain in/be shed from the animal, the concentration of agent in any specific organs, etc. Injected im into the near term fetus; half-life of active hormone (estradiol) is about 90 minutes.

7a. Please indicate whether or not the laboratory personnel and CompMed staff have been informed of these hazards and have received training on proper handling techniques.

Laboratory Personnel		**CompMed Staff**	
x	Yes		Yes
	No	x	No Not needed. They are never in contact with the agent or its by-products

If not, please contact the Environmental Health and Safety Office to schedule the proper training.

8. Please provide any other relevant information regarding the agent (e.g., unique containment recommendations): N/A

2. Please provide specific information about the agent:

Complete name
(Include strain for microorganisms): β-estradiol-3 benzoate

Dose and frequency of administration: 1ml in saline / ethanol X1

Concentration: 2 µg

Route: IM Duration of exposure: 6-24 hours

How long will the animal be maintained after administration? 6-24 hours

Animal species: NHP Estimated animal weight: 700-900 grams

3. Is the agent excreted or shed by the animal?

_____ YES *(Indicate the type of excreta and estimated quantity per day)*

X NO

4. Are there documented human risks from exposure to the agent? (Risks may be determined from the MSDS or from references found in the Biosafety Manual.)

X YES *(Complete Questions 4a-4e)* _____ NO *(Skip to Question 5)*

4a. Indicate the route(s) of human exposure:

X Inhalation
X Ingestion
_____ Other (describe below):
X Contact
_____ Parenteral

4b. Describe the risks associated with the agent (mutagen, teratogen, etc.):

Cancers of the female reproductive tract are often due to exposure to endogenous estradiol and thus chronic exposure is likely also associated with increased risk of cancer.

4c. Indicate the acutely toxic/infectious dose in humans (in mg/kg or number of organisms):

Chronic exposure can induce menstrual irregularities and elicit estrogenic effects in males. Only PI staff is exposed to the agent in raw form.

4d. Describe any genetic changes to the organism and their suspected effects:

N/A

4e. Describe the symptoms of exposure:

Exposure would have to be long term.

4f. Describe the first aid methods to be taken in the case of exposure:

Flush the area with water and contact Occ Health

4g. Indicate all personal protection required:

x	Lab coat/dedicated clothing	____	Apron
x	Gloves	____	Face shield
____	Goggles	____	Respirator
X	Other (describe below):		

Face mask

5. Are there risks to other animals in the room or in the animal facility?

____ YES *(Complete Questions 5a-5d)* x NO *(Skip to Question 6)*

5a. Describe the risk to other animals:

5b. Indicate the route of animal exposure:

5c. Describe all methods that will be used to contain the risk factor:

5d. Are special animal care requirements necessary?

____ YES *(Describe below)* ____ NO

6. Are special waste or carcass disposal requirements necessary?

____ YES *(Describe below)* x NO

7. Briefly describe the procedure(s) for administration of the agent and how the agent is metabolized and/or excreted. Include how long the agent is expected to remain in/be shed from the animal, the concentration of agent in any specific organs, etc. Injected im into the near term fetus; half-life of active hormone (estradiol) is about 90 minutes.

7a. Please indicate whether or not the laboratory personnel and CompMed staff have been informed of these hazards and have received training on proper handling techniques.

	Laboratory Personnel		**CompMed Staff**
x	Yes	____	Yes
____	No	x	No Not needed. They are never in contact with the agent or its by-products

If not, please contact the Environmental Health and Safety Office to schedule the proper training.

8. Please provide any other relevant information regarding the agent (e.g., unique containment recommendations): N/A

EVMS
Eastern Virginia Medical School

December 16, 2017

Gerald J. Pepe, Ph.D.
Chair, Department of Physiological Sciences
Eastern Virginia Medical School
[redacted]
Norfolk, Virginia 23507

Dear Dr. Pepe:

The Institutional Animal Care and Use Committee has reviewed your request to amend the protocol entitled, ***Regulation of Fetal-placental Development in the Primate*** **(IACUC #15-009). The following amendment to the protocol has been approved via the facilitated review (FR) process:**

> **Permission to ascertain the level of skeletal muscle capillary density in preterm baboon fetuses treated or untreated with estradiol (E2) and estradiol benzoate. Maternal baboons will be fasted overnight prior to the *in utero* intramuscular (IM) injection procedure. Estradiol (10 µg) and estradiol benzoate (10 µg) in 0.2 mL of 5% pharmaceutical-grade ethanol/sesame oil (n=4 animals) or vehicle alone (n=4 animals) will be injected IM into the rump or shoulder of the fetus on days 123-129 ± 2 days of gestation. Ultrasound will be used to aid the injection procedure. Day 130 is considered preterm. On day 130 ± 2 days, the fetus will be delivered via cesarean section as outlined in the currently approved protocol.**

Although the Division of Comparative Medicine (CompMed) will make every effort to provide housing, husbandry, and veterinary support for your research project, IACUC approval of this amendment request does not guarantee that CompMed will be able to accommodate the work immediately. Available space and resources within the CompMed animal facility are determined by the number of ongoing projects and the animal census at any given time. Please consult with the CompMed management team to confirm availability prior to implementing the approved modification(s).

It is the responsibility of the principal investigator to notify the Committee, in writing, of any deviation from the IACUC-approved protocol. Please note that a change in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian prior to submitting the amendment request to the IACUC for approval.

It is also the responsibility of the principal investigator to provide for regular observation of the health of the research animals and to provide the Division of Comparative Medicine (CompMed) with the name(s) and telephone number(s) of the person(s) who may be contacted and/or take action in the event that the CompMed staff notes an emergency condition with the research animals.

Thank you for your continued cooperation with the Institutional Animal Care and Use Committee.

Office of Research
[redacted]
NORFOLK, VA 23507
p: 757[redacted]
f: 757[redacted]
www.evms.edu

Sincerely,

[redacted]

[redacted] Vice Chair
Institutional Animal Care and Use Committee

[redacted]

cc: [redacted]
Attending Veterinarian
Division of Comparative Medicine

[redacted]
Program Manager
Division of Comparative Medicine

[redacted]
Senior Associate Dean for Research
Institutional Official

April 2010
Updated February 2011; March 2011; April 2012; August 2013

REQUEST TO AMEND A PREVIOUSLY APPROVED IACUC PROTOCOL

Institutional Animal Care and Use Committee (IACUC)

Eastern Virginia Medical School
Office of Research
IACUC Office
[redacted]
Office: 757-[redacted]
Fax: 757-[redacted]

RECEIVED
DEC 7 RECD
12/7/17
[redacted]

FOR OFFICE USE ONLY [redacted]
Date Received: 12/7/17 **Review Method:** ___ FCR / X FR / ___ Administrative (*Personnel Changes Only*)
IBC Approval? X Yes / ___ No **IBC Approval Date:** 7/9/15 **Final Approval Date:** 12/11/17 [redacted]

General Information and Instructions:

1. All requested amendments must be approved by the IACUC **before** they are implemented.
2. Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian **before** the changes are submitted to the IACUC Office.
3. A request to increase the number of animals assigned to a protocol must be approved by the IACUC **before** the animals are ordered.
4. Submit the original signed amendment form to the IACUC Office located in [redacted] and e-mail the MSWord version of the form to the IACUC Administrator.
5. *The IACUC reserves the right to require submission of an IACUC "Initial Review Form" based upon the Committee's assessment of the amendment(s) requested herein.*

I. ADMINISTRATIVE INFORMATION

Protocol Number: 15-009	**Protocol Initial Approval Date:** July 9, 2015
Protocol Title: Regulation of Fetal-placental Development in the Primate	
Principal Investigator: Gerald J Pepe, Ph.D.	
Approved USDA Pain Code Level(s) – *not required for personnel additions only*: **B, C & D**	

II. PERSONNEL

List all personnel to be added to or removed from the protocol. All persons authorized to work on the protocol must have completed the required CITI/LATA and OHSP training modules and must have completed a health/risk assessment with the Occupational Health Department.

Name	Added (+) or Removed (-)	E-mail Address *(additions only)*	CITI/LATA Training Certification Number *(additions only)*	CITI/LATA Training Species Module(s) Completed *(additions only)*	OHSP Training Certification Number *(additions only)*	Occupational Health/ Risk Assessment Date *(additions only)*
1.						
2.						
3.						
Procedure(s) to be performed by personnel addition #1:						
Procedure(s) to be performed by personnel addition #2:						
Procedure(s) to be performed by personnel addition #3:						

III. PROPOSED CHANGES TO THE ORIGINALLY APPROVED PROTOCOL

☐ **A. CHANGE OF SPONSOR/FUNDING SOURCE**

List the new/approved funding source, the project/grant number, the project/grant title (if different from the IACUC protocol title), and the award period.

☐ **B. CHANGE IN PROJECT SITE(S)**

List all new locations (i.e., building and room number(s)) and indicate what procedure(s) will be performed in each new location.

☐ **C. CHANGE OF SPECIES AND/OR STRAIN**

List the species to be added to or removed from the protocol. Please provide the rationale for any species/strain additions and indicate the USDA Pain Category assignment for each new species/strain. Also, list any anticipated changes in veterinary care and/or husbandry due to the new species/strain.

Species and/or strain	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E

☐ **D. CHANGE IN ANIMAL NUMBERS**

List the number of animals to be added to or removed from the protocol. If animals will be added, please provide the justification for the increase and indicate the number of animals to be assigned to each USDA Pain Category.

Species and/or strain	Year (1, 2 or 3)	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E	TOTAL

Will the animals be ordered through CompMed? _____Yes _____ No ***(Identify the source and provide the rationale/justification below.)***

Will the animals require special housing (e.g., specific bedding requirements, isolator cages, special feed, etc.)
_____ Yes ***(Please specify below.)*** _____ No

USDA Pain Code B = *Breeding or holding colony; no animal manipulation.*
USDA Pain Code C = *Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.*
USDA Pain Code D = *Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.*
USDA Pain Code E = *Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.*

[X] **E. CHANGE IN EXPERIMENTAL PROCEDURES AND/OR INSTRUMENTATION**

Describe, in detail, the proposed change(s). *Please indicate potentially painful/distressing effects on the animals as a result of the proposed change(s). Also, please indicate any expected adverse effects and any changes in monitoring, husbandry, housing, early endpoint, and/or experimental endpoint. Finally, please complete the checklist below.*

We request to inject the fetus intramuscularly (IM) under ultrasound and isoflurane/O_2 (nose-cone) anesthesia with 10 µg estradiol and 10 µg estradiol benzoate in 0.2 ml of 5% pharmaceutical-grade ethanol/sesame oil (n = 4 animals) (the agents are approved under the master protocol) or vehicle alone (n = 4 animals) on days 123, 124, 125, 126, 127, 128, and 129 ± 2 days of gestation. Basically, on days 123-129, maternal baboons that have been fasted overnight will be sedated with ketamine and lightly anesthetized with isoflurane/O_2 using a nose-cone mask. The amniotic fluid/placenta/fetus will be identified by ultrasound. At this stage, the fetus is relatively large/easy to visualize and can be moved up against the uterine wall. Once localized, a 3 inch 23-gauge needle will be inserted through the uterine wall into the fetal rump or shoulder and estradiol injected. The needle will be removed and fetal heart rate will be checked to assure stability. Baboon mothers will then be returned to their cages. On day 130 +/- 2 days, the animal will be prepped for surgery as outlined in the approved protocol and the fetus delivered will be delivered via cesarean section.

The injection procedure does not require analgesia as it mimics the approved amniocentesis procedure. No change to the anesthesia regimen is requested. Any distress to the fetus can be monitored via ultrasound following injection. No adverse effects should arise to the mother after the injections. Any observed abnormal bleeding or changes in vital signs will be noted and the Attending Veterinarian (AV) will be contracted to determine an appropriate course of action.

Please check all that apply to the proposed change(s). Complete the required IACUC Attachment and submit it along with the amendment form.	
Biohazardous Agents: ____ **Yes** __X__ **No** (e.g., recombinant DNA, RNA, all tissue or cell samples, laboratory-induced infection, cultured pathogens)	Complete and submit **Attachment D**
Radioisotopes, *in vivo*: ____ **Yes** __X__ **No**	Complete and submit **Attachment D**
Chemical Agents: ____ **Yes** __X__ **No** (e.g., known or suspected chemical hazards, mutagens, or teratogens)	Complete and submit **Attachment D**
Stress or Prolonged Restraint: ____ **Yes** __X__ **No**	Complete and submit **Attachment C**
Food and/or Water Deprivation: ____ **Yes** __X__ **No**	Complete and submit **Attachment C**
Surgical Procedures: ____ **Yes** __X__ **No** (i.e., single survival, multiple survival, or non-survival) No change to the currently approved surgical procedure is proposed.	Complete and submit **Attachment E**

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

Antibody Production: ____ Yes __X__ No	Complete and submit **Attachment F**
Toxicity Testing (LD50): ____ Yes __X__ No	Complete and submit **Attachment G**
Lasers or Penetrating Electromagnetic Radiation: ____ Yes __X__ No	
Collection of Tissues, Cells, or Organs: ____ Yes __X__ No (Please explain below.)	
Tumor Transplantation or Induction: ____ Yes __X__ No	
Special Diet: ____ Yes __X__ No (Please explain below.)	
Other: N/A (Please specify below.)	

[X] **F. CHANGE IN ANESTHESIA, THERAPEUTICS, AND/OR EXPERIMENTAL AGENTS**

	Dose (mg/kg)	Route of Administration	Frequency of Administration	
Sedatives/Tranquilizers				
Anesthetics - General				
Anesthetics - Local				
				Length of Administration
Analgesics				
Antibiotics				
Miscellaneous				

***USDA Pain Code B** = Breeding or holding colony; no animal manipulation.*
***USDA Pain Code C** = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.*
***USDA Pain Code D** = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.*
***USDA Pain Code E** = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.*

EXPERIMENTAL AGENTS:

Agent: __Estradiol (E_2); 10 µg_____ **Agent Vehicle:** _pharmaceutical-grade ethanol/sesame oil (5%)__
Route/Site: __IM to the fetus_____ **Volume per administration:** __0.2ml_____
Frequency of administration: _____SID for 7 days = 7 injections_____
Expected side effects and/or changes in animal behavior: ___No adverse effects from the injections are expected_____

Agent: __Estradiol Benzoate; 10 µg___ **Agent Vehicle:** _pharmaceutical-grade ethanol/sesame oil (5%) __
Route/Site: __IM to the fetus_____ **Volume per administration:** __0.2ml_____
Frequency of administration: _____SID for 7 days = 7 injections_____
Expected side effects and/or changes in animal behavior: ___ No adverse effects from the injections are expected_____

Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _____

INVESTIGATOR ASSURANCES:

X	*I understand that it is my responsibility as the Principal Investigator to ensure that all personnel who work on this protocol are adequately trained. That training may be provided by me, an experienced member of the investigative staff, the Attending Veterinarian, a qualified Division of Comparative Medicine (CompMed) staff member, or an appropriate outside source. I hereby affirm that each person added to this protocol by way of this amendment request has been adequately trained to perform the procedure(s) indicated above. Any person who has not been adequately trained will be so trained prior to performing the procedure(s).*
X	*No animal involved in this project will be subjected to discomfort, pain, or distress, unless it is unavoidable in the conduct of scientifically valuable research and approval of such has been approved by the IACUC.*
X	*I agree to comply with all federal and institutional policies governing the use of animals used in this project.*

PRINCIPAL INVESTIGATOR:

Printed Name: Gerald J. Pepe, PhD

Signature:	**Date: 12/6/2017**

***USDA Pain Code B** = Breeding or holding colony; no animal manipulation.*
***USDA Pain Code C** = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.*
***USDA Pain Code D** = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.*
***USDA Pain Code E** = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.*

EXPERIMENTAL AGENTS:

Agent: _____Estradiol_____ **Agent Vehicle:** ___ ethanol/sesame oil _____
Route/Site: _____IM to fetus_____ **Volume per administration:** _____0.2ml_____
Frequency of administration: _____SID for 7 days = 7 injections_____
Expected side effects and/or changes in animal behavior: ____No adverse effects from the injections are expected

Agent: ___Estradiol Benzoate_____ **Agent Vehicle:** __ ethanol/sesame oil _____
Route/Site: _____IM to fetus_____ **Volume per administration:** __0.2ml_____
Frequency of administration: _____SID_for 7 days = 7 injections_____
Expected side effects and/or changes in animal behavior: ___ No adverse effects from the injections are expected

Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _____

INVESTIGATOR ASSURANCES:

X	*I understand that it is my responsibility as the Principal Investigator to ensure that all personnel who work on this protocol are adequately trained. That training may be provided by me, an experienced member of the investigative staff, the Attending Veterinarian, a qualified Division of Comparative Medicine (CompMed) staff member, or an appropriate outside source. I hereby affirm that each person added to this protocol by way of this amendment request has been adequately trained to perform the procedure(s) indicated above. Any person who has not been adequately trained will be so trained prior to performing the procedure(s).*
X	*No animal involved in this project will be subjected to discomfort, pain, or distress, unless it is unavoidable in the conduct of scientifically valuable research and approval of such has been approved by the IACUC.*
X	*I agree to comply with all federal and institutional policies governing the use of animals used in this project.*

PRINCIPAL INVESTIGATOR:

Printed Name: Gerald J Pepe, PhD

Signature: Gerald Pepe **Date:** 12/6/2017

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

APPROVAL SIGNATURES:	
VETERINARY PRE-REVIEW: *Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian before the changes are submitted to the IACUC for review.* **Attending Veterinarian Printed Name:** **Attending Veterinarian Signature:**	Primary reviewer approval also serves as vet pre-review [redacted] 12/15/17 **Date:**
FINAL IACUC APPROVAL: *All revisions must be approved by the IACUC prior to implementation.* **IACUC Chair or Vice Chair Printed Name:** [redacted] **IACUC Chair or Vice Chair Signature:**	12/19/17 **Date:**

***USDA Pain Code B** = Breeding or holding colony; no animal manipulation.*
***USDA Pain Code C** = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.*
***USDA Pain Code D** = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.*
***USDA Pain Code E** = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.*

EVMS
Eastern Virginia Medical School

February 25, 2016

Gerald J. Pepe, Ph.D.
Chair, Department of Physiological Sciences
Eastern Virginia Medical School
[redacted]
Norfolk, Virginia 23507

Dear Dr. Pepe:

The Institutional Animal Care and Use Committee has reviewed your amendment request (*i.e., permission to use ketamine alone [250 ml NaCl IV bag, 500 mg, IV] instead of ketofol [ketamine:propofol] to anesthetize prepubertal and postpubertal offspring undergoing IVGTT without muscle biopsy to ascertain the potential impact of propofol; the IVGTT procedure will be performed every 6 months, as outlined in the currently approved protocol)* to the protocol entitled, ***Regulation of Fetal-placental Development in the Primate*** **(IACUC #15-009). The amendment to the protocol has been approved via the facilitated review (FR) process.**

Although the Division of Comparative Medicine (CompMed) will make every effort to provide housing, husbandry, and veterinary support for your research project, IACUC approval of this amendment request does not guarantee that CompMed will be able to accommodate the work immediately. Available space and resources within the CompMed animal facility are determined by the number of ongoing projects and the animal census at any given time. Please consult with the CompMed management team to confirm availability prior to implementing the approved modification(s).

It is the responsibility of the principal investigator to notify the Committee, in writing, of any deviation from the IACUC-approved protocol. Please note that a change in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian prior to submitting the amendment request to the IACUC for approval.

It is also the responsibility of the principal investigator to provide for regular observation of the health of the research animals and to provide the Division of Comparative Medicine (CompMed) with the name(s) and telephone number(s) of the person(s) who may be contacted and/or take action in the event that the CompMed staff notes an emergency condition with the research animals.

Thank you for your continued cooperation with the Institutional Animal Care and Use Committee.

Sincerely,

[redacted]
Chair
Institutional Animal Care and Use Committee

Office of Research

[redacted]

[redacted]

[redacted]

cc: [redacted]
Attending Veterinarian
Division of Comparative Medicine

[redacted]
Project Manager
Division of Comparative Medicine

[redacted]
Senior Associate Dean for Research
Institutional Official

EVMS
Eastern Virginia Medical School

February 8, 2016

Gerald J. Pepe, Ph.D.
Professor and Chair

[redacted] Chair
Institutional Animal Care and Use Committee
Eastern Virginia Medical School
Norfolk, VA 23507

Dear [redacted]

I would like to have the appended research protocol (briefly outlined below) amended to my approved IACUC protocol entitled "Regulation of Fetal-Placental Development in the Primate" (#15-009). Briefly, we are/have been approved to study the effect of estrogen *in utero* on programming fetal and maternal vascular endothelial development/function as well as insulin signaling mechanisms in skeletal muscle and thus vascular function and glucose homeostasis in adulthood. Accordingly, with the exception of cesarean section (i.e. intra-abdominal surgery) we are approved to perform physiologic studies (e.g. iv glucose tolerance tests without or with biopsy of vastus lateralis skeletal muscle) in adult pregnant baboons and baboon offspring sedated with ketamine (0.1 mg/kg BW/min) : propofol (0.2 mg/kg BW/min). In previous years, iv glucose tolerance tests (without a skeletal muscle biopsy) were performed sequentially in offspring at approximately 1, 2 and 3 years of age (prior to puberty) and at 4-8 years of age (after puberty) under ketamine anesthesia. Results of our more recent iv GTT studies in prepubertal offspring completed under ketamine:propofol (ketofol) anesthesia, however appear to differ from those performed previously using only ketamine anesthesia. Interestingly, a recent paper in rats suggests that propofol anesthesia may induce insulin resistance whereas others have previously shown beneficial effects of propofol alone on glucose tolerance in monkeys. Accordingly, I am requesting to perform using ketamine anesthesia alone an iv GTT (no muscle biopsy) in prepubertal and postpubertal offspring in which we have already performed an iv GTT with ketofol (ketamine:propofol) to ascertain potential impact of propofol. As indicated we will not perform a muscle biopsy in these proposed studies using anesthesia with ketamine alone. Also, I want to point out that because we are approved to perform iv GTT under ketamine:propofol in offspring with advancing age, the proposed amendment does not request and thus will not increase the number of experimental iv GTT procedures. We are simply requesting to perform the next iv GTT (without muscle biopsy) under ketamine alone.

Finally, we are requesting that this amendment be reviewed using an expedited process. The IACUC's time and effort in reviewing this amendment is most appreciated.

Sincerely,

Gerald J. Pepe, Ph.D.
Professor and Chair

GJP/[redacted]

[redacted]
P.O. BOX 1980
NORFOLK, VA 23501-1980
ph. 757[redacted]
fax 757[redacted]
www.evms.edu

April 2010
Updated February 2013; March 2013; April 2012; August 2013

REQUEST TO AMEND A PREVIOUSLY APPROVED IACUC PROTOCOL

Institutional Animal Care and Use Committee (IACUC)

FEB 12 2016

Eastern Virginia Medical School
Office of Research

IACUC Office
[redacted]

Office: 757-[redacted]
Fax: 757[redacted]

FOR OFFICE USE ONLY [redacted]
Date Received: 2-12-16 **Review Method:** ___ FCR / X FR / ___ Administrative (*Personnel Changes Only*)
IBC Approval? X Yes / ___ No **IBC Approval Date:** 2/15/16 **Final Approval Date:** 2/15/16 [redacted]

General Information and instructions:

1. All requested amendments must be approved by the IACUC before they are implemented.
2. Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian before the changes are submitted to the IACUC Office.
3. A request to increase the number of animals assigned to a protocol must be approved by the IACUC before the animals are ordered.
4. Submit the original signed amendment form to the IACUC Office located in [redacted] and e-mail the MSWord version of the form to the IACUC Administrator.
5. *The IACUC reserves the right to require submission of an IACUC "Initial Review Form" based upon the Committee's assessment of the amendment(s) requested herein.*

I. ADMINISTRATIVE INFORMATION

Protocol Number: 15-009	**Protocol Initial Approval Date: 7/9/2015**
Protocol Title: Regulation of fetal-placental development in the primate	
Principal Investigator: Gerald J Pepe PhD	
Approved USDA Pain Code Level(s) – *not required for personnel additions only*: **B, C, & D**	

II. PERSONNEL

List all personnel to be added to or removed from the protocol. All persons authorized to work on the protocol must have completed the required CITI/LATA and OHSP training modules and must have completed a health/risk assessment with the Occupational Health Department.

Name	Added (+) or Removed (-)	E-mail Address *(additions only)*	CITI/LATA Training Certification Number *(additions only)*	CITI/LATA Training Species Module(s) Completed *(additions only)*	OHSP Training Certification Number *(additions only)*	Occupational Health/ Risk Assessment Date *(additions only)*
1.						
2.						
3.						
Procedure(s) to be performed by personnel addition #1:						
Procedure(s) to be performed by personnel addition #2:						
Procedure(s) to be performed by personnel addition #3:						

III. PROPOSED CHANGES TO THE ORIGINALLY APPROVED PROTOCOL

☐ **A. CHANGE OF SPONSOR/FUNDING SOURCE**

List the new/approved funding source, the project/grant number, the project/grant title (if different from the IACUC protocol title), and the award period.

☐ **B. CHANGE IN PROJECT SITE(S)**

List all new locations (i.e., building and room number(s)) and indicate what procedure(s) will be performed in each new location.

☐ **C. CHANGE OF SPECIES AND/OR STRAIN**

List the species to be added to or removed from the protocol. Please provide the rationale for any species/strain additions and indicate the USDA Pain Category assignment for each new species/strain. Also, list any anticipated changes in veterinary care and/or husbandry due to the new species/strain.

Species and/or strain	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E

☐ **D. CHANGE IN ANIMAL NUMBERS**

List the number of animals to be added to or removed from the protocol. If animals will be added, please provide the justification for the increase and indicate the number of animals to be assigned to each USDA Pain Category.

Species and/or strain	Year (1, 2 or 3)	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E	TOTAL

Will the animals be ordered through CompMed? _____Yes _____ No ***(identify the source and provide the rationale/justification below.)***

Will the animals require special housing (e.g., specific bedding requirements, isolator cages, special feed, etc.)
_____ Yes ***(Please specify below.)*** _____ No

***USDA Pain Code B** = Breeding or holding colony; no animal manipulation.*
***USDA Pain Code C** = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.*
***USDA Pain Code D** = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.*
***USDA Pain Code E** = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.*

[X] **E. CHANGE IN EXPERIMENTAL PROCEDURES AND/OR INSTRUMENTATION**

Describe, in detail, the proposed change(s). *Please indicate potentially painful/distressing effects on the animals as a result of the proposed change(s). Also, please indicate any expected adverse effects and any changes in monitoring, husbandry, housing, early endpoint, and/or experimental endpoint. Finally, please complete the checklist below.*

We request to use ketamine alone as an anesthesia for IVGTT test with NO biopsy instead of ketofol (i.e., ketamine:propofol) as currently approved. Animals will be sedated IM with ketamine (10-15mg/kg), weighed and legs shaved for catheter placement as already approved. IV drip of Ketamine/NaCl alone will maintain sedation for the duration of the test. ~ 45 minutes start to finish. Timed blood samples will be taken at currently approved intervals (BL00, BL0, T1, T3, T5, T10, T20 and T30) Animal will recover on the table until responsive then returned to the cage until fully upright. All parameters of the test will remain as currently approved.

IVGTT with no muscle biopsy will be performed using ketamine alone in prepubertal and postpubertal offspring in which an IVGTT with ketofol has already been performed to ascertain the potential impact of propofol. Because we are approved to perform IVGTT under ketofol in offspring with advancing age, this proposed amendment does not request and thus will not increase the number of experimental IVGTT procedures. We are simply requesting to perform the next IVGTT without muscle biopsy under ketamine alone.

Please check all that apply to the proposed change(s). Complete the required IACUC Attachment and submit it along with the amendment form.	
Biohazardous Agents: ____ Yes __x__ No (e.g., recombinant DNA, RNA, all tissue or cell samples, laboratory-induced infection, cultured pathogens)	Complete and submit **Attachment D**
Radioisotopes, *in vivo*: ____ Yes __x__ No	Complete and submit **Attachment D**
Chemical Agents: ____ Yes __x__ No (e.g., known or suspected chemical hazards, mutagens, or teratogens)	Complete and submit **Attachment D**
Stress or Prolonged Restraint: ____ Yes __x__ No	Complete and submit **Attachment C**
Food and/or Water Deprivation: ____ Yes __x__ No	Complete and submit **Attachment C**
Surgical Procedures: ____ Yes __x__ No (i.e., single survival, multiple survival, or non-survival)	Complete and submit **Attachment E**
Antibody Production: ____ Yes __x__ No	Complete and submit **Attachment F**
Toxicity Testing (LD50): ____ Yes __x__ No	Complete and submit **Attachment G**
Lasers or Penetrating Electromagnetic Radiation: ____ Yes __x__ No	
Collection of Tissues, Cells, or Organs: ____ Yes __x__ No (Please explain below.)	
Tumor Transplantation or Induction: ____ Yes __x__ No	
Special Diet: ____ Yes __x__ No	

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

(Please explain below.)	
Other : N/A (Please specify below.)	

[X] F. CHANGE IN ANESTHESIA, THERAPEUTICS, AND/OR EXPERIMENTAL AGENTS

	Dose (mg/kg)	Route of Administration	Frequency of Administration
Sedatives/Tranquilizers			
Anesthetics - General			
Ketamine in 250ml NaCl IV bag	500mg	IV	At IVGTT test with NO biopsy, every 6 months
Anesthetics - Local			

				Length of Administration
Analgesics				
Antibiotics				
Miscellaneous				

EXPERIMENTAL AGENTS:

Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _____
_____

Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____
Frequency of administration: _____

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

Expected side effects and/or changes in animal behavior: _____

_____

Agent: _____ **Agent Vehicle:** _____

Route/Site: _____ **Volume per administration:** _____

Frequency of administration: _____

Expected side effects and/or changes in animal behavior: _____

_____

INVESTIGATOR ASSURANCES:

☐	*I understand that it is my responsibility as the Principal Investigator to ensure that all personnel who work on this protocol are adequately trained. That training may be provided by me, an experienced member of the investigative staff, the Attending Veterinarian, a qualified Division of Comparative Medicine (CompMed) staff member, or an appropriate outside source. I hereby affirm that each person added to this protocol by way of this amendment request has been adequately trained to perform the procedure(s) indicated above. Any person who has not been adequately trained will be so trained prior to performing the procedure(s).*
☐	*No animal involved in this project will be subjected to discomfort, pain, or distress, unless it is unavoidable in the conduct of scientifically valuable research and approval of such has been approved by the IACUC.*
☐	*I agree to comply with all federal and institutional policies governing the use of animals used in this project.*

PRINCIPAL INVESTIGATOR:

Printed Name:

Signature: **Date:**

APPROVAL SIGNATURES:

VETERINARY PRE-REVIEW: *Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian before the changes are submitted to the IACUC for review.*

Attending Veterinarian Printed Name: [redacted]

Attending Veterinarian Signature: **Date:2-9-2016**

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.
USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

INVESTIGATOR ASSURANCES:	
☑	*I understand that it is my responsibility as the Principal Investigator to ensure that all personnel who work on this protocol are adequately trained. That training may be provided by me, an experienced member of the investigative staff, the Attending Veterinarian, a qualified Division of Comparative Medicine (CompMed) staff member, or an appropriate outside source. I hereby affirm that each person added to this protocol by way of this amendment request has been adequately trained to perform the procedure(s) indicated above. Any person who has not been adequately trained will be so trained prior to performing the procedure(s).*
☑	*No animal involved in this project will be subjected to discomfort, pain, or distress, unless it is unavoidable in the conduct of scientifically valuable research and approval of such has been approved by the IACUC.*
☑	*I agree to comply with all federal and institutional policies governing the use of animals used in this project.*

PRINCIPAL INVESTIGATOR:	
Printed Name: Gerald J. Pepe, Ph.D.	
Signature: Gerald J. Pepe	**Date:** 2/9/16

APPROVAL SIGNATURES:	
VETERINARY PRE-REVIEW: *Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian before the changes are submitted to the IACUC for review.* **Attending Veterinarian Printed Name:**	
Attending Veterinarian Signature:	**Date:**
FINAL IACUC APPROVAL: *All revisions must be approved by the IACUC prior to implementation.* **IACUC Chair or Vice Chair Printed Name:** [redacted] **IACUC Chair or Vice Chair Signature:**	**Date:** March 2 2016

USDA Pain Code B = Breeding or holding colony; no animal manipulation.
USDA Pain Code C = Procedures involving no or momentary slight pain or distress for which no pain-relieving drugs are used.
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USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

EVMS
Eastern Virginia Medical School

September 1, 2016

Gerald J. Pepe, Ph.D.
Chair, Department of Physiological Sciences
Eastern Virginia Medical School
[redacted]
Norfolk, Virginia 23507

Dear Dr. Pepe:

The Institutional Animal Care and Use Committee reviewed your request to amend the protocol entitled, ***Regulation of Fetal –placental Development in the Primate*** **(IACUC #15-009)**, at its September 1, 2016 meeting. **The following amendments to the protocol were approved:**

1. **permission to change the Phenylephrine dosage from 1 µg/kg/min IV for 15 minutes to 2 µg/kg/min IV for 15 minutes to study the impact of estrogen *in utero* on cardiovascular function and blood flow in maternal and adolescent baboons, and**
2. **addition of the following sustained-release analgesic agents: Buprenorphine SR™ (0.2 mg/kg) and Meloxicam SR™ (0.6 mg/kg) for all surgical procedures.**

Although the Division of Comparative Medicine (CompMed) will make every effort to provide housing, husbandry, and veterinary support for your research project, IACUC approval of this amendment request does not guarantee that CompMed will be able to accommodate the work immediately. Available space and resources within the CompMed animal facility are determined by the number of ongoing projects and the animal census at any given time. Please consult with the CompMed management team to confirm availability prior to implementing the approved modification(s).

It is the responsibility of the Principal Investigator to notify the Committee, in writing, of any deviation from the IACUC-approved protocol. Please note that a change in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian prior to submitting the amendment request to the IACUC for approval.

It is also the responsibility of the Principal Investigator to provide for regular observation of the health of the research animals and to provide the Division of Comparative Medicine (CompMed) with the name(s) and telephone number(s) of the person(s) who may be contacted and/or take action in the event that the CompMed staff notes an emergency condition with the research animals.

Thank you for your continued cooperation with the Institutional Animal Care and Use Committee.

Sincerely,

[redacted]

Institutional Animal Care and Use Committee

[redacted]

cc: [redacted]
Attending Veterinarian
Division of Comparative Medicine

[redacted]
Project Manager
Division of Comparative Medicine

[redacted]
Senior Associate Dean for Research
Institutional Official

EVMS
Eastern Virginia Medical School

August 23, 2016

[redacted]
Institutional Animal Care and Use Committee
Eastern Virginia Medical School
Norfolk, VA 23507

Dear [redacted]:

I would like to have the appended research protocol and postoperative analgesia amended to my approved IACUC protocol entitled "Regulation of Fetal-Placental Development in the Primate" (#15-009). The two amendments requested are described in detail in the attached IACUC amendment form: [1] Deletion of studies using 1 µg/kg/min phenylephrine and add infusion of phenylephrine at a dose of 2 µg/kg/min for 15 min followed by infusion of phenylephrine at 5 µg/kg/min for 15 min (already approved to infuse 5 µg dose) to study the impact of estrogen on vascular function in maternal and adolescent baboons. [2] Sustained release (SR) medications have been developed and studies show that such formulations provide analgesia for up to 72 hours. Accordingly, we propose adoption of the SR medications Buprenorphine SR and Meloxicam SR for all surgical procedures.

Finally, I am requesting that this amendment be reviewed using an expedited process. The IACUC's time and effort in reviewing this amendment is most appreciated.

Sincerely,

Gerald J. Pepe, Ph.D.
Professor and Chair

GJP/[redacted]

Physiological Sciences
[redacted]
P.O. BOX 1980
NORFOLK, VA 23501-1980
[redacted]
www.evms.edu

REQUEST TO AMEND A PREVIOUSLY APPROVED IACUC PROTOCOL

Institutional Animal Care and Use Committee (IACUC)

Eastern Virginia Medical School
Office of Research
IACUC Office
[redacted]
Office: 757-[redacted]
Fax: 757[redacted]

FOR OFFICE USE ONLY [redacted]
Date Received: 8/12/16 **Review Method:** X FCR / ___ FR / ___ Administrative (*Personnel Changes Only*)
IBC Approval? X Yes / ___ No **IBC Approval Date:** 9/15 **Final Approval Date:** 9/16

General Information and Instructions:

1. All requested amendments must be approved by the IACUC **before** they are implemented.
2. Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian **before** the changes are submitted to the IACUC Office.
3. A request to increase the number of animals assigned to a protocol must be approved by the IACUC **before** the animals are ordered.
4. **Submit the original signed amendment form to the IACUC Office located in** [redacted]**, and e-mail the MSWord version of the form to the IACUC Administrator.**
5. ***The IACUC reserves the right to require submission of an IACUC "Initial Review Form" based upon the Committee's assessment of the amendment(s) requested herein.***

I. ADMINISTRATIVE INFORMATION

Protocol Number: 15-009	**Protocol Initial Approval Date:** 7/9/2015
Protocol Title: Regulation of Fetal-placental Development in the Primate	
Principal Investigator: GJ Pepe	
Approved USDA Pain Code Level(s) – *not required for personnel additions only*: **B, C &D**	

II. PERSONNEL

List all personnel to be added to or removed from the protocol. ***All persons authorized to work on the protocol must have completed the required CITI/LATA and OHSP training modules and must have completed a health/risk assessment with the Occupational Health Department.***

Name	Added (+) or Removed (-)	E-mail Address *(additions only)*	CITI/LATA Training Certification Number *(additions only)*	CITI/LATA Training Species Module(s) Completed *(additions only)*	OHSP Training Certification Number *(additions only)*	Occupational Health/ Risk Assessment Date *(additions only)*
1.						
2.						
3.						
Procedure(s) to be performed by personnel addition #1:						
Procedure(s) to be performed by personnel addition #2:						
Procedure(s) to be performed by personnel addition #3:						

III. PROPOSED CHANGES TO THE ORIGINALLY APPROVED PROTOCOL

☐ **A. CHANGE OF SPONSOR/FUNDING SOURCE**

List the new/approved funding source, the project/grant number, the project/grant title (if different from the IACUC protocol title), and the award period.

☐ **B. CHANGE IN PROJECT SITE(S)**

List all new locations (i.e., building and room number(s)) and indicate what procedure(s) will be performed in each new location.

☐ **C. CHANGE OF SPECIES AND/OR STRAIN**

List the species to be added to or removed from the protocol. Please provide the rationale for any species/strain additions and indicate the USDA Pain Category assignment for each new species/strain. Also, list any anticipated changes in veterinary care and/or husbandry due to the new species/strain.

Species and/or strain	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E

☐ **D. CHANGE IN ANIMAL NUMBERS**

List the number of animals to be added to or removed from the protocol. If animals will be added, please provide the justification for the increase and indicate the number of animals to be assigned to each USDA Pain Category.

Species and/or strain	Year (1, 2 or 3)	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E	TOTAL

Will the animals be ordered through CompMed? _____Yes _____ No ***(Identify the source and provide the rationale/justification below.)***

Will the animals require special housing (e.g., specific bedding requirements, isolator cages, special feed, etc.)
_____ Yes ***(Please specify below.)*** _____ No

USDA Pain Code B = *Breeding or holding colony; no animal manipulation.*
USDA Pain Code C = *Procedures involving no or momentary/slight pain or distress for which no pain-relieving drugs are used.*
USDA Pain Code D = *Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.*
USDA Pain Code E = *Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.*

X

E. CHANGE IN EXPERIMENTAL PROCEDURES AND/OR INSTRUMENTATION

Describe, in detail, the proposed change(s). *Please indicate potentially painful/distressing effects on the animals as a result of the proposed change(s). Also, please indicate any expected adverse effects and any changes in monitoring, husbandry, housing, early endpoint, and/or experimental endpoint. Finally, please complete the checklist below.*

This amendment requests two changes to our current approved IACUC protocol #15-009

1. Change the dose of phenylephrine used to study impact of estrogen in utero on cardiovascular function/blood flow in maternal and adolescent baboons.

2. Change post-operative pain control medications for all surgical procedures performed on this protocol.

Change dose of phenylephrine: Currently we are approved to administer phenylephrine iv at a dose of 1 µg/kg body weight/min for 15 minutes followed by infusion for 15 minutes of phenylephrine at a dose of 5 µg/kg/min and assess cardiovascular function (e.g. heart rate; blood pressure) and brachial artery blood flow using noninvasive Doppler ultrasound. Studies conducted to date show that infusion of phenylephrine at 1 µg/kg/min had no effect on cardiovascular function and blood flow in baboon adolescents born to mothers untreated (i.e. controls) or treated during the second half of gestation with aromatase inhibitor letrozole or treated early in pregnancy with estradiol. In contrast, as anticipated, the 5 µg dose was effective. It is important to ascertain whether treated/untreated animals will differ in their response to a lower/e.g. minimal dose and accordingly we are requesting deletion of studies using 1 µg/kg/min phenylephrine and employ infusion of phenylephrine at a dose of 2 µg/kg/min for 15 min followed by infusion of phenylephrine at 5 µg/kg/min for 15 min. There is no expected change in adverse effect to the animal from this change in dose particularly since no adverse effects were noted following infusion of phenylephrine at 5 µg/kg/min. However, if any adverse effects are noted the AV will be notified immediately and the infusion stopped.

Change post-operative pain control medications: Sustained release (SR) medications have been developed and studies show that such formulations provide analgesia for up to 72 hours. Accordingly, we propose adoption of the following use of SR medications for all surgical procedures including maternal cesarean section of pregnant baboons and biopsy of skeletal muscle in baboon offspring.

Buprenorphine SR: Buprenorphine SR will be administered ONCE SQ at a dose of 0.2 mg/kg body weight immediately upon completion of surgery (i.e. animal still anesthetized).

Meloxicam SR: If animal requires additional medication 72 hours post-surgery based on observations by Comp Med staff and/or [redacted], Meloxicam SR will be administered ONCE SQ at a dose of 0.6 mg/kg body weight.

As always, CompMed staff will be notified if an animal shows signs postoperative pain. The Attending or on call Veterinarian will be contacted to determine if additional pain control medication is needed.

We request permission to retain our current postoperative pain control plans (buprenorphine + ketoprofen) in case sustained release products for some unforeseen reasons are/become unavailable.

Please check all that apply to the proposed change(s). Complete the required IACUC Attachment and submit it along with the amendment form.	
Biohazardous Agents: ____ Yes ___x_ No (e.g., recombinant DNA, RNA, all tissue or cell samples, laboratory-induced infection, cultured pathogens)	Complete and submit **Attachment D**
Radioisotopes, *in vivo*: ____ Yes __x__ No	Complete and submit **Attachment D**
Chemical Agents: ____ Yes x____ No (e.g., known or suspected chemical hazards, mutagens, or teratogens)	Complete and submit **Attachment D**
Stress or Prolonged Restraint: ____ Yes _x___ No	Complete and submit **Attachment C**

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USDA Pain Code D = Procedures involving a degree of pain or distress for which appropriate anesthesia, analgesics, or tranquilizing drugs are used.
USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

Food and/or Water Deprivation: ____ Yes __x__ No	Complete and submit **Attachment C**
Surgical Procedures: ____ Yes __x__ No (i.e., single survival, multiple survival, or non-survival)	Complete and submit **Attachment E**
Antibody Production: ____ Yes _x___ No	Complete and submit **Attachment F**
Toxicity Testing (LD50): ____ Yes _x___ No	Complete and submit **Attachment G**
Lasers or Penetrating Electromagnetic Radiation: ____ Yes __x__ No	
Collection of Tissues, Cells, or Organs: ____ Yes _x___ No (Please explain below.)	
Tumor Transplantation or Induction: ____ Yes _x___ No	
Special Diet: ____ Yes _x___ No (Please explain below.)	
Other : N/A (Please specify below.)	

X **F. CHANGE IN ANESTHESIA, THERAPEUTICS, AND/OR EXPERIMENTAL AGENTS**

	Dose (mg/kg)	Route of Administration	Frequency of Administration	Length of Administration
Sedatives/Tranquilizers				
Anesthetics - General				
Anesthetics - Local				
Analgesics				
Meloxicam SR	0.6mg/kg	SQ	Once PRN	PRN under AV advisement
Buprenex SR	0.2mg/kg	SQ	Once upon completion of surgery and for biopsy	Q72 then PRN under AV advisement
Antibiotics				
Miscellaneous				

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EXPERIMENTAL AGENTS:

Agent: Phenylephrine **Agent Vehicle:** sterile saline
Route/Site: IV **Volume per administration:** 2 µg/kg/min over 15min
Frequency of administration: Pre-pubertal and post-pubertal; 4 times over the life span of the animal, not to exceed once a month
Expected side effects and/or changes in animal behavior: Sudden marked increase in blood pressure. Not to be expected at this low dose but animals are monitored for HR, BP and respiratory rate throughout the experiment.

Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _____

Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _____

INVESTIGATOR ASSURANCES:

[x]	*I understand that it is my responsibility as the Principal Investigator to ensure that all personnel who work on this protocol are adequately trained. That training may be provided by me, an experienced member of the investigative staff, the Attending Veterinarian, a qualified Division of Comparative Medicine (CompMed) staff member, or an appropriate outside source. I hereby affirm that each person added to this protocol by way of this amendment request has been adequately trained to perform the procedure(s) indicated above. Any person who has not been adequately trained will be so trained prior to performing the procedure(s).*
[x]	*No animal involved in this project will be subjected to discomfort, pain, or distress, unless it is unavoidable in the conduct of scientifically valuable research and approval of such has been approved by the IACUC.*
[x]	*I agree to comply with all federal and institutional policies governing the use of animals used in this project.*

PRINCIPAL INVESTIGATOR:

Printed Name: Gerald J Pepe, PhD

Signature:	**Date:**

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USDA Pain Code E = Procedures involving a degree of pain or distress for which no drugs are used. SCIENTIFIC JUSTIFICATION IS REQUIRED.

EXPERIMENTAL AGENTS:

Agent: Phenylephrine **Agent Vehicle:** sterile saline
Route/Site: IV **Volume per administration:** 2 µg/kg/min over 10-15min
Frequency of administration: Pre-pubertal and post pubertal
Expected side effects and/or changes in animal behavior: Sudden marked increase in blood pressure. Not to be expected at this low dose but animals are monitored for HR, BP and respiratory rate throughout the experiment.

Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _____

Agent: _____ **Agent Vehicle:** _____
Route/Site: _____ **Volume per administration:** _____
Frequency of administration: _____
Expected side effects and/or changes in animal behavior: _____

INVESTIGATOR ASSURANCES:

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X	*No animal involved in this project will be subjected to discomfort, pain, or distress, unless it is unavoidable in the conduct of scientifically valuable research and approval of such has been approved by the IACUC.*
X	*I agree to comply with all federal and institutional policies governing the use of animals used in this project.*

PRINCIPAL INVESTIGATOR:

Printed Name: Gerald J Pepe, PhD

Signature: [signature] **Date:** Aug 22, 2016

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Attending Veterinarian Printed Name: [redacted] M.S., DVM **Attending Veterinarian Signature:** [redacted]	**Date:** 8-10-2016
FINAL IACUC APPROVAL: *All revisions must be approved by the IACUC prior to implementation.* **IACUC Chair or Vice Chair Printed Name:** [redacted] **IACUC Chair or Vice Chair Signature:** [redacted]	**Date:** Sept. 7, 2016

Digitally signed by [redacted]
DN: cn=[redacted], o=EVMS / SoBran, Inc., ou=Comparative Medicine Division, email=[redacted] c=US
Date: 2016.08.10 09:49:13 -04'00'

[redacted]

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EVMS
Eastern Virginia Medical School

November 5, 2015

Gerald J. Pepe, Ph.D.
Chair, Physiological Sciences
Eastern Virginia Medical School
[redacted]
Norfolk, Virginia 23507

Dear Dr. Pepe:

The Institutional Animal Care and Use Committee reviewed your request to amend the protocol entitled, ***Regulation of Fetal-placental Development in the Primate*** **(IACUC #15-009)**, at its November 5, 2015 meeting. **The following amendment to the protocol was approved:**

> **Permission to treat pregnant baboons as follows: (1) letrozole (115 µg/kg BW/0.1 ml sesame oil, SC) on days +/- 110-120 of gestation; (2) estradiol 17β (5 µg/kg BW, IV bolus) on day +/- 119 24h or 48h prior to surgery; and (3) estradiol 17β-3 benzoate (115 µg/kg, SC) on day +/- 119 once for the 24h treatment group and twice for the 48h treatment group. Following treatment, the placenta/fetus will be delivered @ 6h or 24h (6, 24h treatment group) on day 119/120 or on day 121 (48h treatment group). In the 48h treatment group, the mother will receive a second SC injection of estradiol on day 120 24h after the first injection. Surgery will be performed as outlined in the currently approved protocol.**

Although the Division of Comparative Medicine (CompMed) will make every effort to provide housing, husbandry, and veterinary support for your research project, IACUC approval of this amendment request does not guarantee that CompMed will be able to accommodate the work immediately. Available space and resources within the CompMed animal facility are determined by the number of ongoing projects and the animal census at any given time. Please consult with the CompMed management team to confirm availability prior to implementing the approved modification(s).

It is the responsibility of the Principal Investigator to notify the Committee, in writing, of any deviation from the IACUC-approved protocol. Please note that a change in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian prior to submitting the amendment request to the IACUC for approval.

It is also the responsibility of the Principal Investigator to provide for regular observation of the health of the research animals and to provide the Division of Comparative Medicine (CompMed) with the name(s) and telephone number(s) of the person(s) who may be contacted and/or take action in the event that the CompMed staff notes an emergency condition with the research animals.

Thank you for your continued cooperation with the Institutional Animal Care and Use Committee.

Sincerely,

[redacted]
Institutional Animal Care and Use Committee

[redacted]

cc: [redacted]
Attending Veterinarian
Division of Comparative Medicine

[redacted]
Project Manager
Division of Comparative Medicine

[redacted]
Senior Associate Dean for Research
Institutional Official

EVMS
Eastern Virginia Medical School

Gerald J. Pepe, PhD
Professor and Chair

October 6, 2015

[redacted] Chair
Institutional Animal Care and Use Committee
Eastern Virginia Medical School
Norfolk, VA 23507

OCT 7 2015

Dear [redacted]

I would like to have the appended research protocol (briefly outlined below) amended to my approved IACUC protocol entitled "Regulation of Fetal-Placental Development in the Primate" (#15-009). In the existing protocol, we are approved to treat pregnant baboons with letrozole between days 100 and 175 of gestation to block estrogen production and determine impact on fetal and placental development including ontogeny of vascular endothelial cell growth factor (VEGF) by endothelial cells in skeletal muscle and placental structure, including development of microvilli in fetal/placental tissues from baboons delivered at day 175 of gestation by cesarean section. Another groups of baboons is treated with letrozole and estradiol chronically (days 100-175) to restore estrogen levels. We have also been approved to perform studies in which estrogen is injected directly into the fetus to determine whether estrogen has a relatively rapid (6-24 hours) effect on signalling mechanisms and production of proteins such as VEGF. It has also become clear to us that we also need to examine placental/fetal parameters in animals in which estrogen is suppressed for a relatively short period of time, i.e. 10 days vs long term 60 days. Therefore, we are requesting that we be approved to treat pregnant baboons with letrozole (115 µg/kg BW/0.1 ml sesame oil; sc) on days 110-120 of gestation and inject the mother on day 119 with an iv bolus of free 17β-estradiol (5 µg/kg BW/ml saline:5% ethanol) and a sc injection of 17β-estradiol-3 benzoate (115 µg/kg BW/0.1 ml sesame oil) or steroid vehicle and deliver the placenta/fetus 6, 24 or 48 hours later on day 120 (6, 24 h treatment) or day 121 (48 hour treatment). In the latter group, the mother will receive a second sc injection of 17β-estradiol-3-benzoate on day 120 (i.e. 24 hours after the first injection). All baboons will be injected and cesarean sections performed as outlined in our approved protocol. We were previously approved and thus have performed similar studies in the past. The IACUC's time and effort in reviewing this amendment are most appreciated.

Sincerely,

Gerald J. Pepe, Ph.D.
Professor and Chairman

Physiological Sciences
[redacted]
P.O. BOX 1980
NORFOLK, VA 23501-1980
tel: 757[redacted]
fax: 757[redacted]
www.evms.edu

April 2010
Updated February 2011; March 2011; April 2012; August 2013

REQUEST TO AMEND A PREVIOUSLY APPROVED IACUC PROTOCOL

OCT 7 2015

Institutional Animal Care and Use Committee (IACUC)

Eastern Virginia Medical School
Office of Research
IACUC Office
[redacted]
Office: 757[redacted]
Fax: 757-[redacted]

FOR OFFICE USE ONLY [redacted]
Date Received: 10/7/15 **Review Method:** X FCR / ____ FR / ____ Administrative (*Personnel Changes Only*)
IBC Approval? X Yes / ____ No **IBC Approval Date:** 9/15 **Final Approval Date:** 11/5/15

General Information and Instructions:

1. All requested amendments must be approved by the IACUC **before** they are implemented.
2. Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian **before** the changes are submitted to the IACUC Office.
3. A request to increase the number of animals assigned to a protocol must be approved by the IACUC **before** the animals are ordered.
4. Submit the original signed amendment form to the IACUC Office located in [redacted], and e-mail the MS Word version of the form to the IACUC Administrator.
5. ***The IACUC reserves the right to require submission of an IACUC "Initial Review Form" based upon the Committee's assessment of the amendment(s) requested herein.***

I. ADMINISTRATIVE INFORMATION

Protocol Number: 15-009	**Protocol Initial Approval Date: July 9, 2015**
Protocol Title: Regulation of Fetal-placental Development in the Primate	
Principal Investigator: Gerald J. Pepe, Ph.D.	
Approved USDA Pain Code Level(s) – *not required for personnel additions only*: **B, C, and D**	

II. PERSONNEL

List all personnel to be added to or removed from the protocol. All persons authorized to work on the protocol must have completed the required CITI/LATA and OHSP training modules and must have completed a health/risk assessment with the Occupational Health Department.

Name	Added (+) or Removed (-)	E-mail Address *(additions only)*	CITI/LATA Training Certification Number *(additions only)*	CITI/LATA Training Species Module(s) Completed *(additions only)*	OHSP Training Certification Number *(additions only)*	Occupational Health/ Risk Assessment Date *(additions only)*
1.						
2.						
3.						
Procedure(s) to be performed by personnel addition #1:						
Procedure(s) to be performed by personnel addition #2:						
Procedure(s) to be performed by personnel addition #3:						

III. PROPOSED CHANGES TO THE ORIGINALLY APPROVED PROTOCOL

☐ **A. CHANGE OF SPONSOR/FUNDING SOURCE**

List the new/approved funding source, the project/grant number, the project/grant title (if different from the IACUC protocol title), and the award period.

☐ **B. CHANGE IN PROJECT SITE(S)**

List all new locations (i.e., building and room number(s)) and indicate what procedure(s) will be performed in each new location.

☐ **C. CHANGE OF SPECIES AND/OR STRAIN**

List the species to be added to or removed from the protocol. Please provide the rationale for any species/strain additions and indicate the USDA Pain Category assignment for each new species/strain. Also, list any anticipated changes in veterinary care and/or husbandry due to the new species/strain.

Species and/or strain	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E

☐ **D. CHANGE IN ANIMAL NUMBERS**

List the number of animals to be added to or removed from the protocol. If animals will be added, please provide the justification for the increase and indicate the number of animals to be assigned to each USDA Pain Category.

Species and/or strain	Year (1, 2 or 3)	USDA Pain Code B	USDA Pain Code C	USDA Pain Code D	USDA Pain Code E	TOTAL

Will the animals be ordered through CompMed? _____Yes _____ No ***(Identify the source and provide the rationale/justification below.)***

Will the animals require special housing (e.g., specific bedding requirements, isolator cages, special feed, etc.)
_____ Yes ***(Please specify below.)*** _____ No

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X **E. CHANGE IN EXPERIMENTAL PROCEDURES AND/OR INSTRUMENTATION**

Describe, in detail, the proposed change(s). *Please indicate potentially painful/distressing effects on the animals as a result of the proposed change(s). Also, please indicate any expected adverse effects and any changes in monitoring, husbandry, housing, early endpoint, and/or experimental endpoint. Finally, please complete the checklist below.*

We would like to treat pregnant baboons on days +/- 110-120 of gestation with letrozole (115 µg/kg BW/0.1 ml sesame oil; sc) and inject the mother on day +/-119 with an iv bolus of estradiol 17β (5 µg/kg BW) and sc estradiol 17β-3 benzoate (115ug/kg) then deliver the placenta/fetus 6, 24 on day 119/120 (6, 24 h treatment) or day 121 (48 hour treatment). In the latter group, the mother will receive a second sc injection of estradiol on day 120 (i.e. 24 hours after the first injection).
Surgery will be conducted as outlined in the approved protocol with no change.

Letrozole, estradiol 17β, and estradiol 17β-3 benzoate are already on the approved protocol and all necessary attachments are on file.

Please check all that apply to the proposed change(s). Complete the required IACUC Attachment and submit it along with the amendment form.	
Biohazardous Agents: ____ Yes __x__ No (e.g., recombinant DNA, RNA, all tissue or cell samples, laboratory-induced infection, cultured pathogens)	Complete and submit **Attachment D**
Radioisotopes, *in vivo*: ____ Yes __x__ No	Complete and submit **Attachment D**
Chemical Agents: ____ Yes __x__ No (e.g., known or suspected chemical hazards, mutagens, or teratogens)	Complete and submit **Attachment D**
Stress or Prolonged Restraint: ____ Yes __x__ No	Complete and submit **Attachment C**
Food and/or Water Deprivation: ____ Yes __x__ No	Complete and submit **Attachment C**
Surgical Procedures: ____ Yes __x__ No (i.e., single survival, multiple survival, or non-survival)	Complete and submit **Attachment E**
Antibody Production: ____ Yes __x__ No	Complete and submit **Attachment F**
Toxicity Testing (LD50): ____ Yes __x__ No	Complete and submit **Attachment G**
Lasers or Penetrating Electromagnetic Radiation: ____ Yes __x__ No	
Collection of Tissues, Cells, or Organs: ____ Yes __x__ No (Please explain below.)	
Tumor Transplantation or Induction: ____ Yes __x__ No	
Special Diet: ____ Yes __x__ No	

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(Please explain below.)	
Other : N/A (Please specify below.)	

[X] **F. CHANGE IN ANESTHESIA, THERAPEUTICS, AND/OR EXPERIMENTAL AGENTS**

	Dose (mg/kg)	Route of Administration	Frequency of Administration	
Sedatives/Tranquilizers				
Anesthetics - General				
Anesthetics - Local				
				Length of Administration
Analgesics				
Antibiotics				
Miscellaneous				

EXPERIMENTAL AGENTS:

Agent: ____Estradiol 17β_____ Agent Vehicle: __ Saline/5%ethanol _____
Route/Site: _____IV_____ Volume per administration: _<0.2 ml_____
Frequency of administration: ____Once 24 or 48 hours before surgery_____
Expected side effects and/or changes in animal behavior: _None anticipated_____
_____

Agent: __ Estradiol 17β-3 benzoate _____ Agent Vehicle: _ Sesame oil (sterile)_____
Route/Site: _____SC_____ Volume per administration: __0.2 to 1.0 ml_____
Frequency of administration: ____Once for 24h treatment group or twice for 48h group_____
Expected side effects and/or changes in animal behavior: __None anticipated_____

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X	*I understand that it is my responsibility as the Principal Investigator to ensure that all personnel who work on this protocol are adequately trained. That training may be provided by me, an experienced member of the Investigative staff, the Attending Veterinarian, a qualified Division of Comparative Medicine (CompMed) staff member, or an appropriate outside source. I hereby affirm that each person added to this protocol by way of this amendment request has been adequately trained to perform the procedure(s) indicated above. Any person who has not been adequately trained will be so trained prior to performing the procedure(s).*
X	*No animal involved in this project will be subjected to discomfort, pain, or distress, unless it is unavoidable in the conduct of scientifically valuable research and approval of such has been approved by the IACUC.*
X	*I agree to comply with all federal and institutional policies governing the use of animals used in this project.*

PRINCIPAL INVESTIGATOR:

Printed Name: Gerald J. Pepe, Ph.D.

Signature: [signature] **Date:** 10/06/2015

APPROVAL SIGNATURES:

PRE-VETERINARY REVIEW: *Changes in methodology, agents, surgical procedures, or any aspect of the protocol that affects the health, husbandry, and/or well-being of the animals must be pre-reviewed by the Attending Veterinarian before the changes are submitted to the IACUC for review.*

Attending Veterinarian Printed Name: [redacted]

Attending Veterinarian Signature: [redacted] **Date:** 10/7/15

FINAL IACUC APPROVAL: *All revisions must be approved by the IACUC prior to implementation.*

IACUC Chair or Vice Chair Printed Name: [redacted]

IACUC Chair or Vice Chair Signature: [redacted] **Date:** Nov 16 2015

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FINAL IACUC APPROVAL: *All revisions must be approved by the IACUC prior to implementation.* **IACUC Chair or Vice Chair Printed Name:** **IACUC Chair or Vice Chair Signature:**	**Date:**

***USDA Pain Code B** = Breeding or holding colony; no animal manipulation.*
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