FILE COPY

[redacted]

From: [redacted]
Sent: Monday, January 4, 2021 11:29 AM
To: [redacted]
Cc: [redacted]
Subject: PAM Protocol #18-006

To: [redacted] Principal Investigator
CC: [redacted] IACUC Administrator
Protocol #: 18-006
Title: *Regulation of Fetal-placental Development in the Primate*
Protocol member present: [redacted]

Dear [redacted]

On Jan. 4, 2021 a routine animal program review of the activities approved under the protocol identified above was conducted by [redacted] CompMed Program Manager, on behalf of the EVMS Institutional Animal Care and Use Committee (IACUC).

One animal, 26741, was observed during blood collection, CGS and Estradiol injections. With respect to the procedures observed under this protocol, all procedures observed were performed as approved in the protocol. Please commend your staff for the attention to detail, the professional manner in which the animal activities were conducted, and the humane manner in which the animals were handled.

Successful reviews such as this provide clear evidence of institutional regulatory compliance, as dictated by the Animal Welfare Act and the Public Health Service. Thank you and your staff for your gracious hospitality and your support of our institution's commitment to quality care and progressive research. Congratulations on a job well done!

A copy of this memo note will be maintained with the IACUC Administration.

Thank you,

[redacted]
Project Manager
Division of Comparative Medicine
Eastern Virginia Medical School
[redacted]

Information regarding Research Compliance can be found at http://www.evms.edu/research/office/compliance.html

Information regarding the Institutional Biosafety Committee can be found at http://www.evms.edu/research/office/biosafety.html

EVMS
Eastern Virginia Medical School

FILE COPY

July 11, 2021

[redacted]
Chair, Department of Physiological Sciences
Eastern Virginia Medical School
[redacted]
Norfolk, Virginia 23507

Dear [redacted]

The requested revision to the closure form noting the expiration of your protocol, entitled ***Regulation of Fetal-Placental Development in the Primate*** **(IACUC #18-006)**, was reviewed by the Institutional Animal Care and Use Committee via Designated Member Review (DMR). **The protocol is officially closed and will be archived.**

If you wish to reinstitute this research project, please complete a new Initial Review Form, and submit the original form to the IACUC Office located in [redacted]

All IACUC forms are available on the IACUC website at https://[redacted]
or from the IACUC Office.

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]
Program Manager
Division of Comparative Medicine

[redacted]
Vice Dean for Research
Institutional Official

Research
[redacted]
Norfolk, VA 23507
TEL [redacted]
FAX

EVMS

View xForm - IACUC Annual Review/Closure Form

Annual Review/Closure Form

Annual Review/Closure Form Data Entry - Submitted 07/06/2021 10:35 PM ET by [redacted]

Project Information and Instructions

10.10 Creating User

[redacted]

10.20

Annual Review/Closure Form

The IACUC Annual Review/Closure Form is used by the Principal Investigator to request continuation of an IACUC-approved protocol for an additional year or to request closure or termination of an IACUC-approved protocol. ***An Annual Review Form must be submitted every year, EVEN if no animals have been used during the reporting period.*** *The Principal Investigator must provide a narrative description of the results obtained from the animals used to date.* ***The narrative must provide sufficient detail to allow the IACUC to determine whether or not the continued use of animals is justified.*** *A Closure Form must be submitted when* ***(1)*** *all research will not be completed before the protocol 3-year expiration date and a new IACUC application will be submitted for continuance of the project (i.e. Project Expired);* ***(2)*** *all research has been completed prior to the 3-year expiration date and no further work will be performed (i.e. Project Completed), or* ***(3)*** *the Principal Investigator wants to end all research prior to the protocol 3-year expiration date (i.e. Project Terminated).*

Submit this form 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.***

10.30 Project Title:

Regulation of Fetal-Placental Development in the Primate

10.40 Principal Investigator:

[redacted]

10.50 Protocol Number:

18-006

10.60 Project Status: ***What is the current status of this project?***

Project Expired:

10.63 Closure: Expiration Date
06/06/2021

FINACIAL CONFLICT OF INTEREST

20.10 FCOI Instructions

If any of the activities described in this IACUC-approved 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.

20.20

Have you, other family members, or any other person responsible for the design, conduct, or reporting of this research project:

20.21
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?

No

20.22 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?
No

20.23 Received intellectual property rights (e.g., patents, copyrights, and/or royalty income from such rights)?
No

20.24 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.
No

20.25 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?
No

20.30 FUNDING SOURCE(S)

20.31 Is this project funded?
Yes

20.32 Funding Source Table

If applicable, please list all current and/or potential sources of funding for this protocol. Click SAVE on the right hand side after each row.

Funding Source:	NIH
Funding Status:	Approved
IACUC Approval Verification Letter:	No

AR/Closure Form Questions

30.10
METHOD

Has the experimental methodology, the number of approved animals, the experimental and/or therapeutic agents, the anesthesia, and/or the euthanasia used in this project changed since the last time the project was reviewed?

No

30.11 Summarize all approved amendments to the protocol during the reporting period and list the approval date for each entry.
No changes since the last review

30.20 ANIMAL USAGE

30.30 Species 1
Non-Human Primate

30.31 Species 1: Number of Approved Animals
206

30.32 # of used to date:
53

30.35 Species 2

N/A

30.50 MULTIPLE SURVIVAL SURGERY

30.51 Is the current protocol approved for multiple survival surgery?
Yes

30.60
PERSONNEL

Have there been any changes in personnel performing the animal experiments since the last time the project was reviewed?

No

30.70
NARRATIVE DESCRIPTION

Please provide a narrative describing the results obtained from the animals used to date. The narrative should include morbidity rates resulting from EXPECTED and UNEXPECTED ADVERSE EVENTS encountered in the conduct of the protocol. Examples of morbidity that should be reported include, but are not limited to, infection, dehiscence of the incision site, coma due to induced hypoglycemia, diarrhea, fighting, cannibalism, overgrooming, and/or failed procedures. These adverse events should be reported, whether or not they were treated by the Attending Veterinarian and/or resulted in mortality. Expected morbidity and mortality occurring as prescribed in the protocol (e.g., euthanasia, non-survival surgery, limb amputation) should not be included. Include information only associated with adverse events.

Two (2) adult female baboons (#26741 and #28768) were studied during pregnancy per approved protocols. Baboon #26741 spontaneously delivered a fetus pre-term. The fetus did not survive. The mother suffered no complications from the delivery and is cycling normally. She will return to the breeding colony after four (4) normal menstrual cycles. Animal #28768 is currently under treatment with Letrozole and is doing well. Survival surgery for the mother is scheduled at Day 165 of gestation (approximate date = mid-late June, 2021).

[redacted]

MORTALITY RATE for the reporting period: 3.8% (Due to the spontaneous pre-term delivery, the fetus was not expected to survive, and one animal succumbed during treatment with CGS during the 3 year period; 2 animals out of 53 animals total)

MORBIDITY RATE for the reporting period: 1.9% (1 female injured during breeding out of 53 animals total)

AR Multiple Survival Surgery Attachment

40.10
INSTRUCTIONS: The Principal Investigator MUST complete this form if ANY animals assigned to this IACUC-approved protocol have undergone multiple survival surgeries. This attachment must be submitted with the IACUC *Annual Review Submission*.

40.20 The total number of major survival surgeries performed in the PREVIOUS reporting period:

4

40.30 The total number of major survival surgeries performed in the CURRENT reporting period:

0

40.40 The number of animals that have undergone more than one (1) major survival surgery in the PREVIOUS reporting period:

4

40.50 The number of animals that have undergone more than one (1) major survival surgery in the CURRENT reporting period:

0

40.60 Please list all animals that have undergone six (6) major survival surgeries to date and the intended disposition of the animals:

None

40.70 Please list all animals that have undergone more than six (6) major survival surgeries to date and the disposition of the animals:

None

40.80 Please list all surgical complications experienced during the CURRENT reporting period and the outcome of the individual animal(s) that experienced the complications:

No surgeries were performed during the reported period.

Chair Signature
- Submitted 07/13/2021 1:40 PM ET by [redacted]

chair signature

10.10 By entering your password below, you are electronically signing this protocol.

Signed Tuesday, July 13, 2021 1:40:14 PM ET by [redacted]

EVMS
Eastern Virginia Medical School

☐ FILE COPY

June 7, 2018

[redacted]
Chair, Department of Physiological Sciences
Eastern Virginia Medical School
[redacted]
Norfolk, Virginia 23507

Dear [redacted]

Your protocol entitled, ***Regulation of Fetal-Placental Development in the Primate*** **(IACUC #18-006)**, was reviewed by the Institutional Animal Care and Use Committee at its June 7, 2018 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 **April 10, 2019**.

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
[redacted]
www.evms.edu

cc: [redacted]

EASTERN VIRGINIA MEDICAL SCHOOL
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

RECEIVED
MAY 15 2018
OFFICE FOR RESEARCH
EVMS

FOR IACUC USE ONLY:	
IACUC Number: 18-006	Review Date(s): 6/7/18
NOTES: ORIGINAL / FINAL	Final Approval Date: 6/7/18
	Progress Report Due: 4/10/19, 4/10/20

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 #:**	15-009

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):	[redacted]		
Department:	Physiological Sciences		
Mailing Address:	[redacted]		
Office Phone #:		Home or Cell Phone #:	[redacted]
Laboratory Phone #:		E-mail Address:	

Animal Emergency Contact Person:	[redacted]		
Office Phone #:	[redacted]	Home or Cell Phone #:	[redacted]
Laboratory Phone #:		E-mail Address:	

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]	7/1/18	6/30/21

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)		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	IBC Review			

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;
- 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:	[redacted]	Date:	5/14/2018
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	5-15-18
Typed or Printed Name	[redacted]		

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		Date	
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	
Typed or Printed Name	[redacted]		

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	05/15/218
Typed or Printed Name	[redacted]		

IACUC APPROVAL:

IACUC Chair or Designee Signature		Date	
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	[redacted]	Date	6-4-18
Typed or Printed Name	[redacted]		

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	June 12, 2018
Typed or Printed Name	[redacted]		

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 and skeletal muscle. 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 remain to be determined. Thus a major goal of the second series of experiments outlined in this protocol is to elucidate the latter. Interestingly, our new preliminary data suggest that estrogen appears to regulate development of the microvessel network in fetal skeletal muscle which is needed for delivery of glucose and insulin.

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 offspring reared to adulthood. Development of microvessels, 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 placental hormones *in utero* on programming fetal organ systems critical for development of appropriate vascular (e.g. blood pressure/flow) 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	5/01/18	Estrogens/estrogen receptor modulators, etc (24416); embryonic/fetal growth/development (39948); gonad, testis, adrenal glands, adipose tissue, skeletal muscle, placenta, pancreas, etc (31890); menstrual cycle, pre-eclampsia, insulin resistance, fertility, angiogenesis, etc (198287); uterine artery remodeling (80); cesarean section-complications/adverse effects (2781); contraception: (vaginal/cervical pessary-adverse effects (0)); papio/ baboons (687); conscious bleeding (0); animal welfare, animal models, pain/stress/distress/suffering, refine/replace/ reduce, humane endpoint, etc. (1884325). **Citations = 10, 0, 0**	2015-2018 3 yr update
CAB Abstracts Database				
TOXLINE				
BIOSIS Database				
Other: Web of Science (Science Citation Index) Searcher – [redacted]	X	4/30/18	Estrogens/estrogen receptor modulators, etc (26607); embryonic/fetal growth/development (56371); gonad, testis, adrenal glands, adipose tissue, skeletal muscle, placenta, pancreas, etc (41490); menstrual cycle, pre-eclampsia, insulin resistance, fertility, angiogenesis, etc (102936); uterine artery remodeling (8920); reproductive/ developmental biology (40462); cesarean section-adverse effects/complications (6218); contraception (vaginal/ cervical pessary-adverse effects (1)); papio/baboons (1244); conscious bleeding (0); animal welfare, animal models, pain/stress/distress/suffering, refine/replace/ reduce, humane endpoint, etc. (2571439). **Citations = 6, 1, 0**	2015-2018 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) and HD 093070 (2017-2022). DK 093590 will be submitted as a new application to NIH on June 5, 2018 and reviewed for renewal in October with expected start-date in March/April, 2019. The competing renewal of this grant scored a 20th percentile; but was approximately 7% away from the funding line. However, a significant aspect of work/studies developed under the auspices of this grant are still in progress and funded by departmental and/or EVMS sources and data being published and used as supportive rationale/preliminary data for the grant submitted 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" and book chapters. 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 (2015-2018; 3 year update) identified (depending on key word) anywhere from 80 to over 2,571,439 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 either do not have a fetal-placental unit, do not exhibit fetal organ system maturation (e.g. adrenal glands; gonads) as occurs *in utero* in the human and/or exhibit unique patterns of postnatal development not typically noted in the human. Most importantly, the 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 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, 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 which affects up to 15% -20% of all pregnancies, no one has been able to demonstrate cause:effect. Moreover, in 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 submitted a proposal in this regard. Our funded 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 of what happens *in utero* that actually impacts physiological outcomes 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 or those delivered prematurely develop hypertension or insulin resistance/diabetes, respectively. 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 fetal-placental development important for neonatal well-being and they will outline potential therapeutic interventions/ modalities for prevention of hypertension/insulin resistance in adulthood 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 the 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 nonhuman primates/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 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 hemomonochorial and monodiscoid (a single) placenta and a functional fetoplacental unit in which the fetal adrenal gland provides the C_{19}-steroid precursors required for placental estrogen formation. Moreover, baboons and humans share >96% DNA/genetic homology including that for components of the insulin receptor signaling pathway. Because non-primate laboratory animals, e.g. the laboratory rat, do not exhibit hemomonochorial placentation and do not possess a fetoplacental unit for the biosynthesis of hormones such as estrogen and maturation of critical fetal organ systems e.g. 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 and fetal 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 >35 years of baseline data which this laboratory has obtained in pregnant baboons form a critically important basis for the continued use of this animal model and further point 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 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 [redacted]). 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. 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 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 (9) and spontaneously aborted/still birth fetus (~9)	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*) fetuses	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*) Adult females and juveniles	28 adult females and 13 EVMS-born juveniles	28 adult females and 13 EVMS-born juveniles	28 adult females and 14 EVMS-born juveniles	84 adult females and 40 EVMS-born 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**. (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 35 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; placental ischemic diseases) are equally harmful to placental/fetal development and physiologic function in adulthood. Indeed evidence shows that babies born to mothers with these diseases develop insulin resistance which often progresses to diabetes.

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 maternal vascular function 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 and 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 60, 100, 150, 160; day 175 placenta/fetus delivered, euthanized and studied (n = 8)
Group 4: Maternal estradiol daily days 25-59; maternal studies - days 60, 100, 150, 160; day 175, placenta/fetus delivered, euthanized and studied (n = 8)
Group 5: Untreated; maternal studies - days 60,100,150,160; deliver spontaneously; offspring reared and metabolic and vascular function studied pre and post-puberty (n = 8)
Group 6: Maternal estradiol daily on days 25-59; maternal studies - days 60,100,150,160; deliver spontaneously; offspring reared and metabolic and vascular function studied pre and post-puberty (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. skeletal muscle, fat, liver, lung, heart and the pancreas to study expression of proteins that are essential for insulin action/secretion. We also will be examining the impact of estrogen on development and function of the microvessels (microvasculature) in fetal/offspring skeletal muscle important for delivery of glucose and insulin to this tissue. Short term *in vivo* treatment of the fetus with estrogen and *in vitro* studies including incubation of fetal tissues with estradiol or the estradiol receptor agonists and/or inhibitors of downstream signaling molecules are also performed to determine the mechanism of estrogen action. 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 fetal tissues/ microvasculature as well as fetal tissue glucose tolerance/insulin sensitivity are not the result of modification of maternal mechanisms. Finally, offspring born to mothers treated *in utero* with nothing, aromatase inhibitor ± estrogen are raised to adulthood (puberty at 36-48 months of age) and glucose tolerance/insulin action and microvascular development and 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). The procedure is performed during the initial ivGTT to reduce the number of sedations.

Experimental Treatment Groups:

Group 1: Untreated; maternal ivGTT and FMD* day 80 and 90; fetus delivered and studied day 60-120 (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 deliver 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)

Group 8: Fetal estradiol once day 160-175, delivered via c-section 6 to 24 hours post-injection fetus studied. (n=8)

Total number of pregnancies for Study II: N = 64 (8/group x 8 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)	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 ~60-120d 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 **
8	Fetal estradiol (160-175d) 2ug/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 6-24hrs post injection, fetus euthanized

* 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 [redacted] as has occurred over the past 30 years. Thus, approximately 50% of the studies/animal treatments will be performed at the [redacted] 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 continuous 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 no swelling of sexual skin.

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 is < 10% total blood volume/not to exceed 10 ml/kg/month. Animals are returned to their 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 baseline 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 ivGTT 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 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. 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 to the mother under direction of the Attending Veterinarian (AV) 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.

Ultrasound guided fetal injection: On day ~160-175 of gestation under isoflurane anesthesia and ultrasound, baboons are sedated with ketamine (10-15mg/kg), placed on isoflurane via cone mask, placenta/fetus is identified via ultrasound. Once localized, a 3 inch 23 gauge needle is inserted through the uterine wall into the fetal rump or shoulder and estradiol in 1.0ml saline/5% ethanol injected. Needle is removed and fetal heart rate is checked. Baboon mothers are then returned to home cage, monitored to recovery and 6-24 hrs later fetus is delivered via c-section.

Cesarean section: On days ~60 to ~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 disinfecting solution (e.g., Betadine). 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 [redacted] 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 O_2 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 baseline 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 microvessel development in and 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.

Intravenous glucose tolerance tests without muscle biopsy: 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 O_2 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 (0.4 mg/kg) 0.6ml/minute. Once sedated, two baseline 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. **No muscle biopsies will be taken during this ivGTT.**

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 Beuthanasia-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 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. 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. 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 (iSTAT), 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 1-2 and 5 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 acetylcholine (8 µg/kg bw/min) proposed in the baboon, are the average dose infused to humans 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 non-pregnant 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 Attending Aeterinarian.* At least one month later the analyses are repeated but phenylephrine (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.*

STUDY III: CONRAD COLLABERATION PILOT STUDY WITH NON-PREGNANT BABOONS TESTING PROGESTERONE AS DELAY MECANISM IN PREMATURE BIRTH:

We would like to use 6 non-pregnant regularly menstruating female baboons 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 under ketamine/isoflurane anesthesia. This will be done at the early follicular, late follicular and early and late luteal phase of the cycle. [2] Following a one month recovery, under approved sedation we insert a specially designed pessary (4.5cm in diameter by 1.5cm) 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. On day 3-5 (early follicular), 10-12 (late follicular), and 16-18 (early luteal) and 24-26 (late luteal) days after insertion of the pessary, under approved sedation, we will obtain 3ml blood sample, 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. Animal 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 PO will be given PRN. [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. Blood samples (3 ml), a swab of the cervix and conduct a noninvasive 2D ultrasound of the uterus to determine endometrial growth/thickness under ketamine/isoflurane anesthesia. This will be done at the early follicular, late follicular and early and late luteal phase of the cycle as in the other two study groups.

Two ectocervical biopsies will be collected under each of the following conditions for a total of 6 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). 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 Dr. [redacted] who is authorized personnel on the protocol. [redacted] has performed the same procedure in women and has the requisite experience and expertise. The animal is sedated and anesthetized prior to the procedure using ketamine then isoflurane / 0_2 mask. 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.

There will be a washout period between the three experimental treatments. Only a total of 6 baboons will be used. Each animal will act as its own control.

N	Tx Group	Blood Samples	Ultrasound / Swab	Pessary / Injection	Vaginal Biopsy
6	Control	Early & Late follicular, Early & Late luteal	Early & Late follicular, Early & Late luteal		Late luteal
6	Pessary	Early & Late follicular, Early & Late luteal	Early & Late follicular, Early & Late luteal	Early Follicular / removed Late luteal	Late luteal
6	Progesterone Injection	Early & Late follicular, Early & Late luteal	Early & Late follicular, Early & Late luteal	~d2 and d7 of cycle	Late luteal

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. IV for ivGTT without muscle biopsy. 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 Attending 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. Alternative is Buprenorphine SR

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.

Buprenorphine SR: Possible irritation at injection site. Could cause GI upset if used for long period of time, respiratory depression, cardiovascular depression, decreased body temperature, sedation, nausea, vomiting, and diarrhea. AV will be consulted for treatment if this occurs.

Meloxicam SR: Possible irritation at injection site. Other less likely side effects may include abdominal pain, anemia, and edema. AV will be consulted if any of these are observed or perceived.

Fetal injection: minor procedure, possible complications may include fetal death. Fetus is delivered 6-24 hours post injection and if death does occur it should cause no complication to the mother during this short period.

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 Attending 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 Attending 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.

Serotonin: at this low dose, we do not anticipate any change in behavior or long-term physiological effects.

Dextrose (50%): local pain and vein irritation may occur. Diabetic coma, delirium tremors and congested states or pulmonary edema are unlikely but potential consequences. HR and BP are monitored before and after injection. Fetal HR is checked in the case of pregnant baboons.

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 Attending 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 Attending Veterinarian contacted for treatment options

N-nitro-L-arginine methyl ester (L-NAME) 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.

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 has 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, pCO_2, pH etc) and glucose will be determined using iStat 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. CompMed 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 Attending 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, IM to fetus; 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 Attending 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.
Progesterone pessary: Potential change in menstrual cycle, possible loss of appetite. Device could cause discomfort or become dislodged on its own. The Attending Veterinarian will be contacted if any of these occur. We will give Banamine if directed for discomfort.
Hydroxyprogesterone caproate: Possible GI upset or loss of appetite. Irritation at injection site. If either occur we will adjust the diet according to AV direction and address the pain as advised.

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 the PI staff and twice daily by the 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 Attending Veterinarian will be notified. Animal weight is monitored while on study protocol monthly when sedated. Blood chemistry is run 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 Attending 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	III	Anesthetic/sedation	0.4 IV or 10-15mg/kg IM and 0.1mg/kg (with propofol) IV
Euthanasia-D solution	III	Euthanasia	1mg/5kg

Name of the DEA Registrant	[redacted]
DEA Research License Registration Number or Application Confirmation Date	[redacted]
DEA License Effective Date	[redacted]
DEA License Expiration Date	[redacted]

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 Or 0.4mg/kg	IM or IV	Chemical restraint for all protocol blood sampling (1-4 days a week), ultrasound exam, or preoperatively, ivGTT
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, CONRAD study groups
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, Cervical/vaginal Biopsy	Surgery + 2 days BID, SID for vaginal biopsy
Ketoprofen	75mg	PO	Post operatively for surgery and ivGTT w/ biopsy and FMD w/ biopsy and PRN for Pessary placement	Alternative to IM injection when more suitable for the animal in question (2 days BID)
Buprenorphine SR	0.2mg/kg	SQ	Once before C-section and muscle biopsy	Q72hr then PRN under AV advisement
Meloxicam SR	0.6mg/kg	SQ	Once PRN	Under AV advisement
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: 0.2-2.0 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); saline/5% ethanol
Volume per Administration: 0.2 to 1.0 ml
Route of Administration: SQ or IM to fetus
Site of Administration: Abdominal area or back; rump or shoulder of fetus
Frequency of Administration: Administered daily on days ~100-170 of gestation in conjunction with Letrozole to restore estrogen production. Also administered once IM day 160-175 of gestation to fetus to prematurely elevate estrogen levels
List all expected side effects and/or changes in the animal's behavior: None are anticipated

17. Agent: Progesterone 4.1%; 5.5% silicone oil w/w
Agent Vehicle: Silicone pessary device
Volume per Administration: 5.5mg/day
Route of Administration: Local
Site of Administration: Intravaginal
Frequency of Administration: Continuous ~d2 of cycle until removed late luteal phase ~d21-28
List all expected side effects and/or changes in the animal's behavior: There are no expected side effects from the drug itself. Potential change in menstrual cycle, possible loss of appetite. Device could cause discomfort or become dislodged on its own. The attending will be contacted if any of these occur. We will give Banamine if directed for discomfort.

17. Agent: Hydroxyprogesterone caproate
Agent Vehicle: Castor oil
Volume per Administration: 250 mg/mL (1 ml)
Route of Administration: Intramuscular injection (IM)
Site of Administration: Thigh muscle
Frequency of Administration: 2x on day 12 and day 7 after menstruation

List all expected side effects and/or changes in the animal's behavior: Possible GI upset or loss of appetite. Irritation at injection site. If either occur we will adjust the diet according to AV direction and address the pain as advised

17. Agent: Octafluoropropane gas-filled microbubble suspension (MB contrast agent)
Agent Vehicle: 0.9% 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: 1-2 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 Attending 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 Attending Veterinarian contacted for treatment options.

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 Attending Veterinarian consult.

17. Agent: N-nitro-L-arginine methyl ester (L-NAME)
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-NAME 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:

18. 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 housed individually in double cages (two cages joined together) and females are housed individually in a single cage. A male baboon is pair-housed for 2-5 days with a female during her period of estrous for purposes of mating; thus for breeding purposes the male and female share a double cage (two cages joined together). This increases mating contact and has proven to yield higher pregnancy rates with no increase in incidence of altercations and we have had no adverse injuries from this breeding design. Importantly, animals are not stressed as females do become pregnant e.g. ovulate and mate; males produce sperm and mate.

Some adult females are incompatible with others and physical trauma arises, over-grooming occurs, nutritional issues arise (one animal loses weight from not eating), or over-submissive behavior is exhibited. Another reason for singly housing animals is self-injurious behavior (SIB) which can be stressful to a cage mate.

Late gestation pregnant animals expected to deliver spontaneously will be singly housed during late gestation to ensure safe delivery of fetus.

Most animals are at a minimum partially paired allowing liberal touch but may be limited on full range contact. Semiannual reevaluation of the colony attempts to reintegrate singly housed animals. All animals will at a minimum have visual and auditory and olfactory sense of other NHPs.

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:	[redacted]	[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/1 / /
Occupational Health and Safety (OHSP) Training Certification Number	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Occupational Health and Safety Risk Assessment Date (Month / Year)	1/11/18	12/4/17	3/28/18	3/5/18	4/19/18	3/6/18
CITI Training Certification Number and 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 Handling	X	X	X	X	X	
Anesthesia			X			
Surgery	X	X	X	X	X	
Post-Surgical Care			X			
Monitoring	X	X	X	X		
Euthanasia	X		X			

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

[redacted] 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 [redacted] and has been performing surgeries (cesarean section) for 15 years without direct assistance of [redacted]. Although [redacted] 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 [redacted] actually performs all of the animal husbandry, e.g., animal injections; blood sampling etc and also works with CompMed and other laboratory personnel in hand-rearing of baboon neonates per SOP. [redacted] has had significant experience assisting [redacted] 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 [redacted] 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 perform the biopsy procedure [redacted] has performed the same procedure in women and has the requisite experience and expertise to perform the required biopsies.

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 2-4 day 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. [5] **CONRAD study:** Blood samples are collected at early follicular, late follicular, early luteal and late luteal with one additional sample taken during the menstrual cycle. This will occur during the 3 phases of the study with wash out cycles in between. Samples are taken under ketamine sedation using 21g needle volume to be no more than 5mls per sample

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: 2-4 day intervals during gestation from mother; once every other week from adolescents; at 4-5 time points within one cycle of adult females.

23b. **Urine/feces sampling**

Sampling method:

Frequency and duration of sampling:

23c. **Collection of tissues**

All tissues to be collected: **[1]** Kidney, liver, lung, gonads, adrenal, pituitary, pancreas, skeletal muscle, visceral and SQ fat, intestine, heart and uterine samples. **[2]:** Collection of a total of 6 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)

When will tissues be collected (before or after euthanasia)? **[1]:** After euthanasia in both adult sacrificed animals and fetuses following euthanasia at surgery, **[2]:** Late luteal phase in the 3 pilot test groups under Isoflurane / 0_2 anesthesia.

Final disposition of collected tissues: Fixed and/or frozen for experimentation (histopathology)

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 the CompMed staff. Medical problems will be reported to the Attending 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]

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 staff or a 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 the kidney and liver (or others as determined by the Attending Veterinarian) which are to be 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 survival 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 the [redacted] 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:	[redacted] (This is a rare occurrence and all EVMS policies are carried out in consultation with the EVMS AV.)		

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 Attending 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

Attachment B: Nonhuman Primate Enhancement Procedure

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

Protocol #: 18-006

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 singly housed due to elevated possible aggression toward unreceptive females.
   Some adult females are incompatible with others and physical trauma arises, over-grooming occurs, nutritional issues arise (one animal loses weight from not eating), or over-submissive behavior is exhibited. Another reason for singly housing animals is self-injurious behavior (SIB) which can be stressful to a cage mate.
   Late gestation pregnant animals expected to deliver spontaneously will be singly housed during late gestation to ensure safe delivery of fetus.
   Most animals are at a minimum partially paired allowing liberal touch but may be limited on full range contact.
   Semiannual reevaluation of the colony attempts to reintegrate singly housed animals. All animals will at a minimum have visual and auditory and olfactory sense of other NHPs.

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
Protocol #: 18-006

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** [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 0.2 to 2.0 ml 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_ 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
_X_ Gloves
_X_ Goggles
_X_ Other (describe below): Mask
_____ Apron
_____ Face shield
_____ Respirator

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.

The drug is prepared at a concentration of 2 mg/ml sesame oil; animals injected daily 0.2 to 2.0 ml letrozole/kg BW/day subcutaneously; the drug is not shed or excreted by the animal.

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 subcutaneously by PI staff and is not handled by the CompMed 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: _____)

   X Acutely toxic chemical (CAS #: 59-42-7; LD50 in mice: 120 mg/kg; LD50 in rats: 30 mg/kg)

   ____ 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): Phenylephrine

   Dose and frequency of administration: 1-2 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 Contact
   X Ingestion — ____ Parenteral
   ____ Other (describe below):

   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; LD50 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.

The agent will be administered IV over a 30 minute period; the active agent is likely catabolized by the liver and excreted via the 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 intravenously by PI staff and is not handled by the CompMed 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: _____)

____ 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
115 µg/kg BW daily for 70 days in pregnant baboons

Concentration: 2mg/ml

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__ 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 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 metabolized (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.

Daily, estradiol benzoate is injected subcutaneously to pregnant baboons for 35 or 70 days. The agent is first converted to the active hormone estradiol 17β (benzoate is removed) and then catabolized to estrone and glucuronylated and excreted via the 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 subcutaneously by PI staff and is not handled by the CompMed 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

Protocol Number: 18-006

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.

CompMed staff
[redacted] Principal Investigator
[redacted] Research Associate 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 procedures. 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-HCl (10-15mg/kg) IM

4. Briefly describe how the animals will be prepared for surgery.

On days ~60, ~100, ~170 of gestation based on the study group, the animal is sedated with Ketamine-HCl (10-15 mg/kg), intubated and anesthetized with isoflurane/ oxygen. Vitals (e.g., HR, BP, CO2, RR, and temperature) are 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 24 inches in length and IV fluids are administered (~1.6 ml/min over a 90 min period). The animal's abdomen (surgical site) is shaved and scrubbed with alcohol and disinfecting solution (e.g., Betadine). 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 the CompMed staff will be primarily responsible for anesthesia. When CompMed staff is not available, [redacted], [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 the 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 the animal will be monitored for swallowing reflex and response to stimuli before it is extubated. Vitals are monitored until extubation. The animal is then returned to its cage.

The CompMed staff is primarily responsible for immediate anesthesia monitoring. [redacted] will be present also.

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]. If she is unavailable, the CompMed staff will provide post-op care in accordance with IACUC policy and in consultation 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.

The post-op monitoring sheet will be completed by [redacted]. If she is unavailable, a member of the CompMed staff will complete the form. 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) (Alternative to Buprenorphine SR)

Dose and Route: 2mg/kg IM Frequency: At surgery and 2days post-operatively

Post-Operative Duration of Care: 5-7 days of observation (BID for 3days; SID for remainder)

Agent: Ketoprofen (Alternative to Banamine)

Dose and Route: 75mg PO Frequency: 2 days post-operatively

Post-Operative Duration of Care: Monitoring for 5-7days

Agent: Buprenorphine SR

Dose and Route: 0.2mg/kg SQ Frequency: Q72hr then PRN under AV advisement

Post-Operative Duration of Care: 5-7 days of observation (BID for 3 days; SID for remainder)

Agent: Meloxicam SR

Dose and Route: 0.6mg/kg SQ Frequency: Under AV advisement

Post-Operative Duration of Care: 5-7 days of observation (BID for 3days; SID for remainder)

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 fetal-placental 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) animals and animals 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 (C-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 of surgeries 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 (e.g., 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 IACUC #15-009.

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

Please see the surgery log submitted to the IACUC

14b. Identify all previous procedures performed on the animal(s) identified in Question 14a.

Please see the surgery log submitted to the IACUC

14c. List the IACUC protocol number(s) under which the previous procedures were performed.

#15-009 (this submission is a 3-year renewal of 15-009)

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 is studied. Multiple pregnancies also mimic the situation in humans.

15. Surgery Classification 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.

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 X NO The answer is also No; please see non-survival for mother as stated in the surgery procedure outlined below (p. 6, item #4)

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 (e.g., inability to sustain pregnancy, repeated failure to become pregnant, unhealthy uterus). This will be determined in consultation 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 in the CompMed [redacted] surgery suite between 8am and 4pm, M-F

20. Describe the entire surgical procedure.

Once prepped as outlined in #4 (p. 1), 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 Beuthanasia-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 massage 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) or Buprenorphine SR (SQ) 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 Beuthanasia-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 Beuthanasia-D solution and isoflurane gas is elevated to the highest level to ensure cessation. Upon confirmation of death, maternal tissue samples will be taken (i.e., 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 the SOP for rearing neonates. Once the cord is cut, placenta is processed for analysis. The uterus is flushed with saline and sutured. Manual massage 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) or Buprenorphine SR (SQ) 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 the SOP for rearing neonates. Placenta is processed for analysis. The mother is then euthanized by IV injection of Beuthanasia-D solution and isoflurane gas is elevated to the highest level to ensure cessation. Upon confirmation of death, maternal tissue samples will be taken (i.e., 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: 18-006

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.

CompMed staff
[redacted] Principal Investigator
[redacted]
[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 procedures. 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-HCl (10-15mg/kg) IM

4. Briefly describe how the animals will be prepared for surgery.

Briefly, the animal is fasted overnight, and sedated with Ketamine-HCl. The animal is intubated and O_2 is delivered. The backs 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. The animal is monitored for vitals (e.g. HR, BP, CO2, RR, and temperature) by the CompMed staff. The animal's quadriceps are shaved to clear the area for biopsy and scrubbed with alcohol and disinfecting solution (e.g., Betadine). 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 the CompMed staff will be primarily responsible for anesthesia. When the 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 will be kept by the CompMed staff.

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 are removed and the animal will be monitored for swallowing reflex and response to stimuli before it is extubated. Vitals are monitored until extubation. The animal is then returned to its cage.

The CompMed staff is primarily responsible for immediate anesthesia monitoring. [redacted] will be present also.

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] If she is unavailable, the CompMed staff will provide post-op care in accordance with IACUC policy and in consultation 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.

The post-op monitoring sheet will be completed by [redacted] If she is unavailable, a member of the CompMed staff will complete the form. 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) (Alternative to Buprenorphine SR)

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 of observation (BID for 3days; SID for remainder)

Agent: Ketoprofen (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 three (3) muscle biopsy procedures over a course of 15 years (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 of procedures 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 (e.g., 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.**

NOTE: 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 were/will be exposed to minor surgical manipulations.

15. Surgery Classification 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.

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 three (3) muscle biopsy procedures over a course of 15 years (one at ~24-30 months and the 2nd and 3rd 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 in the CompMed [redacted] 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 baseline 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 min. 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 it is warmed via a warming blanket. Samples are taken to measure the 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, the animal is given 1cc (10mg/kg) Iron Dextran IM. Catheters are removed and the animal will be monitored for swallowing reflex and response to stimuli before it is extubated. The animal is then returned to its cage.

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 in the CompMed [redacted] surgery suite between 8am and 4pm, M-F

20. Describe the entire surgical procedure.

At completion of the 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 is inserted through the uterine wall into the amniotic cavity and 10 ml of fluid is collected. Fetal HR is re-checked. This will take place at the completion of the ivGTT to ensure complete sedation of the animal. At completion of the experiment, the animal is given 1cc (10mg/kg) Iron Dextran IM. Catheters are removed and the animal will be monitored for swallowing reflex and response to stimuli before it is extubated. The animal is then returned to its cage.

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: 18-006

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.

CompMed staff
[redacted] Principal Investigator
[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 procedures. 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-HCL (10-15mg/kg) IM

4. Briefly describe how the animals will be prepared for surgery.

Briefly, the animal is fasted overnight and sedated with Ketamine-HCL. The animal is administered isoflurane gas/CO_2 via a cone mask. The perineal area is prepped with antiseptic solution.

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 the CompMed staff will be primarily responsible for anesthesia. When the 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/CO_2 mix via nose cone @ ~ 1-3 flow rate.
Anesthesia monitoring sheets are maintained by the 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 procedure, the animal will be removed from anesthesia and monitored until it is responsive to touch or begins to move on its own. HR and RR will be monitored throughout the procedure until the animal is responsive. The CompMed staff is primarily responsible for immediate anesthesia monitoring. [redacted] will be present also.

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] If she is unavailable, the CompMed staff will provide post-op care in accordance with IACUC policy and in consultation 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.

The post-op monitoring sheet will be completed by [redacted] If she is unavailable, a member of the CompMed staff will complete the form. 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: Once at completion of the procedure and PRN per AV advisement

Post-Operative Duration of Care: SIB for 5days

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**.

The animals are sedated and vaginal biopsies are obtained using a Tischler forcep. There are 3 treatment groups and each animal will act as its own control.

13b. How many surgeries will each animal undergo?

Each animal may undergo up to three (3) vaginal biopsy procedures over the course of the study. One during each treatment group in the late luteal phase of the cycle.

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: I Procedure to be performed: Vaginal Biopsy

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.)**

_____ 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.

Only procedure to be performed

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.

Procedures take place in the CompMed [redacted] surgery suite between 8am and 4pm, M-F

20. Describe the entire surgical procedure.

The animal is sedated and anesthetized prior to the procedure with Ketamine-HCl IM. The animal is administered Isoflurane gas/CO_2 mix via a cone mask and maintained at ~1-3 flow rate. HR and RR are monitored. 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. Anesthesia is turned off, the animal is monitored until it is responsive or begins to move on her own. Flunixin meglumine (Banamine) is given IM for pain management. SIB monitoring is performed for 5 days checking for signs of pain, abnormal bleeding, or change in overall expected behavior.

RC # 18-0016

IACUC Surgery Documentation for Multiple Survival Surgeries

Boon #	sx Date	sx Date	sx Date	sx Date	Total
[redacted]					
'26741	Sept 2016	August 2017			2
No surgical complications					
[redacted]					
28768	Nov 2014				1
No noted complications					

EVMS
Eastern Virginia Medical School

FILE COPY

May 6, 2021

[redacted].
Chair, Department of Physiological Sciences
Eastern Virginia Medical School
[redacted]
Norfolk, Virginia 23507

Dear [redacted]

Your protocol, entitled ***Regulation of Fetal-Placental Development in the Primate*** **(IACUC #21-003)**, was reviewed by the Institutional Animal Care and Use Committee at its May 6, 2021 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 **March 10, 2022**.

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 an 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 situation 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]

Research

[redacted]
NORFOLK, VA 23510
[redacted]
www.evms.edu

Community focus. World impact.

cc: [redacted]
Attending Veterinarian
Division of Comparative Medicine

[redacted]
Program Manager
Division of Comparative Medicine

[redacted]
Vice Dean for Research
Institutional Official

FILE COPY

EVMS

View xForm - IACUC Initial Review Form

IACUC Initial Review Form

IACUC Data Entry
- Submitted 04/12/2021 4:54 PM ET by [redacted]

Instructions

10.10

Welcome to the initial review form. In this form, you will be supplying information for your initial submission of an IACUC protocol or 3 year renewal of an existing IACUC protocol.

A few things of note that are different from the paper version form:

1. This is an interactive form, in that it will guide you to what needs to be completed. If a questions says "(required)" next to it, it MUST be completed before submission of the form. you will be unable to submit the form with any tagged questions not filled.

2. This form will change, add questions and pages, and remove pages and questions, depending on the answers you provide. As such, you may notice that sometimes question or pages numbers seem to skip. This is normal, and means that some question you answered previously determined that you did not need to fill out a question or page. However, if a question or page is there, and with the required tag, then you MUST complete it to be able to submit.

3. Though this form has most of the same questions as the previous initial review form, there are differences, including some new questions or changes as appropriate. Please be sure to FULLY read every question when answering in order to make certain your submission is not delayed.

4. All attachment forms have been incorporated into this xForm. Should your answers show you need to complete an attachment, it will show the attachment towards the end of the form, and you will be required to complete it prior to submission.

5. This form can be filled by more than one person. The upper left link labeled collaborators will allow you to add whomever you wish to the form, in order to assist in completing it. The PI assurances however can ONLY be filled by the PI.

6. You can skip pages in this form to complete later, using the page selection at the top, in the drop down box. However, you will still need to complete all required pages prior to submission, and skipping pages can cause you to lose track of what pages you have completed.

7. Many questions have the option to add a note. This note IS PART OF the official record of the application. However, it is NOT an answer to the question. You must answer the question in the space provided. The Notes option has been allowed in case any question needs clarification and there is no space to place it, or to assist between collaborators. If you have a question that requires IACUC to answer, please contact them directly at IACUC@evms.edu

8. The Form saves responses when a page is advanced, or when the Save for Later button is used. Please be sure to save your work before closing the browser. For tables or cards, there will be a specific save button that should be pressed after EACH row/card to save that information. For pages that can repeat, there will be a specific button to add an additional page, and you may add as many pages as are needed, but be sure to save work for each page before moving on.

Project Header

20.10 Submitter

[redacted]

20.20 Project Title

Regulation of Fetal-Placental Development in the Primate

If the project title is different from the grant title, please list both titles.

20.30 Is this a 3 year renewal of an existing IACUC Protocol?

Yes

20.31 If this is related to another IACUC Protocol, please enter the Protocol Number(s).
18-006

20.40 Species

Non-Human Primate

20.41 List the species information, including the specific strain(s) if applicable, the sex(es), and the age(s) of the animals.
Baboon (Papio anubis/cynocephalus);
Adult female (7-15 yrs) and adult male (8-20 yrs) baboons, as well as offspring born to animals in the colony and studied in the pre- and post-pubertal period and as adults;
Additionally, baboon fetuses will be used for study ranging from gestational day 60 to day 175

20.42 If only using one sex, please provide scientific justification for doing so.
N/A

20.50 Second species, if applicable

No answer provided. *Clicking "No selection" will reset the question.*

20.60 Principal Investigator

[redacted]
Email: [redacted]
Business: Eastern Virginia Medical School (EVMS)
[redacted]
Norfolk, VA 23507
Assistant: (757) [redacted]

20.61 Animal Emergency Contact Person

[redacted]
Email: [redacted]
Business: Eastern Virginia Medical School (EVMS)
[redacted]
Norfolk, VA 23507
Business: 757-[redacted]

20.62 Technical Coordinator
No answer provided.

20.70 Co-Investigators

No answer provided.

20.80 Please list ALL other personnel that will be involved with this project, including volunteers, research assistants, staff, and students. You do not have to list CompMed staff, unless they are taking a significant active role in this protocol, or unless there are *specific* CompMed staff that will be part of this protocol.

[redacted]

20.90 Please choose your Veterinarian.

[redacted] *This should always be the Attending Veterinarian unless otherwise informed by IACUC Administration.*

Project Details

30.10 List all project sites. Please include buildings and rooms.

CompMed Animal Facility, [redacted]
CompMed Animal Facility,

30.20 Expected Project Start Date

06/06/2021

30.21 Expected Project End Date
06/05/2024

30.30 Governmental Funding Sources

Federal Government *Check all that apply*

30.31
Please specify the source(s) of your funding. Also, please state the status of funding for EACH funding source.

NIH HD 093070 (2017-2022); approved
NIH DK 093946 (2020-2025); approved

30.40 Non Governmental Funding Sources.

Campus/Department Funds *Check all that apply*

30.41 Please specify all funding sources and please list the status of funding for EACH funding source.
Departmental Funds: Research Incentive account; approved

30.50 Is an IACUC approval verification letter needed for the funding source(s)?

No

30.51 Approval Verification letter instructions
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.

30.60 Does your protocol involve the use of any hazardous agents?

Entered: 03/30/2021 **By:** [redacted] **Internal:** No

The [redacted] Lab has IBC approval for use of letrozole, which blocks estrogen synthesis.

Yes

30.70
Other Committee Reviews

Please fill in the appropriate information on the table row for agents that the project involves. You will describe one committee/office/agent per row, clicking save at the rightmost column in order to save the the information you filled in.

Make certain to click save on the right after completion of each row.

Project involves/Committee Office	Certification Number or Approval Date	Hazard to
Known or Suspected Chemical Hazards, Mutagens or Teratogens/ EVMS EH&S	IBC approval June 9, 2020	Personnel

Financial Conflict of Interest

40.10

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 @ 446-8480 for assistance.

40.20 Have you, other family members, or any other person responsible for the design, conduct, or reporting of this research project:

40.21 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?
No

40.22 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?
No

40.23 Received intellectual property rights (e.g., patents, copyrights, and/or royalty income from such rights)?
No

40.24 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.
No

40.25 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?
No

Protocol Information

50.10 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.

50.11

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 mom to stay healthy, and 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 in 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, the mother, and the placenta interact and actually communicate with each other via the hormone estradiol. Unfortunately, it is impossible to perform invasive experiments in women 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.

BACKGROUND: Using the baboon as a translational research model for the human, we recently showed that exposure of the mother to a very small increase in estradiol early in pregnancy 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 vascular 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 to determine 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, it 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.

RESEARCH DESIGN: 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 will be collected and studied for aspects of biochemical/physiologic maturation. In other experiments, treated/untreated animals will be delivered near term and neonates will be reared to adulthood. Development of vascular function (e.g., ability to control blood pressure), blood vessel flow, and glucose (sugar) regulation will also be determined as indexes and/or predictions of development of diabetes. 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.

50.20

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.

50.30 Database and computer systems

Enter the information about the database and computer systems you used in the table below. Click save on the right hand side after each row.

50.31

Databases/Computer systems	Date Search Conducted	Keywords/Search Strategy	Time period Covered by the Search
MEDLINE Database	03/10/2021	Searcher: [redacted] estrogens/estrogen receptor modulators, etc(241602); embryonic/fetal growth/development (321625); gonad, testis, adrenal glands, adipose tissue, skeletal muscle, placenta, pancreas, etc (345340); menstrual cycle, pre-eclampsia, insulin resistance, fertility, angiogenesis, etc (1767764); uterine artery remodeling (225); cesarean section-complications/adverse effects(17564); contraception: (vaginal/cervical pessary-adverse effects (279); papio/baboons (14193);conscious bleeding (26); welfare,animal models, pain/stress/distress/suffering, refine/replace/reduce, humane endpoint, etc. (1246689); Citations= 2 (Line 62),1 (Line 69)	2018-2021
Web of Science (Science Citation Index)	03/10/2021	Searcher: [redacted] estrogens/estrogen receptor modulators, etc (26388); embryonic/fetal growth/development (51836); gonad, testis, adrenal glands, adipose tissue, skeletal muscle, placenta, pancreas, etc (41913); menstrual cycle, pre-eclampsia, insulin resistance, fertility, angiogenesis, etc (107993); uterine artery remodeling (9133); cesarean section-complications/adverse effects (6073); contraception: (vaginal/cervical pessary-adverse effects (255); papio/baboons (1020);conscious bleeding (0); welfare,animal models, pain/stress/distress/suffering, refine/replace/reduce, humane endpoint, etc. (3086076); Citations= 1 (line 26)	2018-2021

50.40 Literature and reference sources

Enter the information about the database and computer systems you used in the table below. Click save on the right hand side after each row.

50.41

Literature and reference sources	Date	Keywords/Search Strategy	Time Period covered by the search

50.50 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

50.60 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) and NIH HD 093070 (2017-2022). 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 now funded by NIH DK 093590 (2020-2025), HD 093070 (2017-2022), and departmental sources/research incentives and data being published. 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 (2018-2021; 3 year update) identified (depending on key word) anywhere from <1,000 to over 2,000,000 results; total number >3,955,000. 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 manuscripts/abstracts cited/printed as relevant to the search questions, many 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, they do not exhibit fetal organ system maturation as occurs in utero in the human (e.g., adrenal gland, ovary or testis), and they exhibit unique patterns of postnatal development that impact organ system development, also not typically noted in the human. Most importantly, the 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.

Protocol Narrative

60.10

The narrative must address the following:

60.11 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, 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 diseases, the combined incidence of which affects up to 15% -20% of all pregnancies, no one has been able to demonstrate cause:effect. In 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 occurs later in the pregnancy. Our animal model is ideal for that and we were awarded two NIH grants (HD 093070 and HD 093946) to study UAR regulation and develop a new imaging technique to ascertain compromised UAR early in pregnancy. Our studies are directly applicable to the human and show that too much estrogen early in pregnancy may be a causative factor. Indeed, women in IVF (ART) programs almost always have extremely high levels of estradiol and progesterone in early stages of their pregnancies and the risk of having an IUGR baby or of the mother developing preeclampsia is much higher than normal. Moreover, blood flow to an perfusion of the baboon placenta is virtually identical to that in humans and, thus, the imaging techniques we are using in our model will definitely be able to be used in women. 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 investigators to test that what happens in utero actually impacts the 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 50.31 did not identify any alternative methods/procedures for conduct of studies to examine the role of pregnancy on placental-fetal development and the 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.

60.12 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 vascular, skeletal muscle, and hepatic maturation and function in human pregnancy, as well as the 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 hemomonochorial and monodiscoid placenta and a functional fetoplacental unit, in which the fetal adrenal gland provides the major portion of C19-steroid precursors required for placental estrogen formation. Because non-primate laboratory animals (e.g., the laboratory rat) do not exhibit hemomonochorial placentation and do not possess a fetoplacental unit for the biosynthesis of hormones, such as estrogen, and because their maturation of fetal organ systems occurs primarily postnatally (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 tetra¬hydrocortisone 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 >35 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 50.31 did not identify any alternative methods/procedures for conduct of studies to examine the role of pregnancy on placental-fetal development and the 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 as outlined in Daniel (Biostatistics: A Foundation Analysis in the Health Sciences, 4th Ed., 1987).

60.13 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 organ/vascular development. 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. 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 50.31 did not identify any alternative methods/procedures for conduct of studies to examine the role of pregnancy on placental-fetal development and the 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.

Additionally, under our protocol, we perform multiple surgeries per animal (within the scope of "The Guide") with appropriate care and guidance from the Attending Veterinarian (AV).

60.14 Will the animals be subjected to procedures that may cause more than momentary or slight pain or distress?
Yes

[NOTE: These procedures include environmental, nutritional, or behavioral modifications that increase stress, as well as chronic food or water deprivation.]

60.15 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 50.31 did not identify any alternative methods/ procedures for conduct of studies to examine the role of pregnancy on placental-fetal development and the 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.

60.16 Is the proposed study duplicative of research previously undertaken by the investigator or other scientists?
No

60.18 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.
Throughout the course of conduct of our studies, we have consulted a statistician at EVMS (or at the [redacted]). 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. With n=8/group and pooled estimate of variance 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 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.

NOTE: The IACUC may require a consultation with a statistician.

60.20
USDA Pain levels

B Breeding, conditioning or holding animals for use in teaching, testing, experiments, research or surgery, but not yet used for such purposes; holding colonies fall into here.

C Teaching, research, experiments or tests conducted on animals involving no pain or distress (i.e., euthanizing animals for tissues; observation under normal conditions; positive reward projects

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

E Teaching, research, experiments, surgery or tests conducted involving a degree of pain or distress and for which the use of appropriate anesthetic, analgesic or tranquilizing drugs is NOT used because their use would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery or tests (must be scientifically justified)

60.21 Please Choose all USDA Pain Levels which will be involved in your Protocol
Level B
Level C
Level D

USDA Pain Level B

70.10 Breeding or holding colony protocols where animals do not undergo any manipulation. Unused pups generated during breeding should be included in Level B.

Entered: 04/07/2021 **By:** [redacted] **Internal:** No

The animals assigned to USDA Pain Code Level B include adult male breeders (9) and spontaneously aborted/still birth fetuses (~9); n=18.

70.20 Species
Non-Human Primate

70.30 Year 1
6

70.40 Year 2
6

70.50 Year 3
6

70.60 Total
18

USDA Pain Level C

80.10 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.

Entered: 04/07/2021 **By:** [redacted] **Internal:** No

The animals assigned to USDA Pain Code Level C include fetuses that will be used for experimentation or tissue collection; n=63.

80.20 Species
Non-Human Primate

80.30 Year 1
21

80.40 Year 2
21

80.50 Year 3
21

80.60 Total
63

USDA Pain Level D

90.10 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.

Entered: 04/07/2021 **By:** [redacted] **Internal:** No

The animals assigned to USDA Pain Code Level D include adult females (n=35) and EVMS-born juveniles/neonates (n=40) that will be used for experimentation; n=75.

90.20 Species
Non-Human Primate

90.30 Year 1
25

90.40 Year 2
25

90.50 Year 3
25

90.60 Total
75

Study Procedures

110.10 Please indicate all procedures to be performed in this study

Non-Survival Surgery
Single Major or Minor Survival Surgery
Multiple Major Survival Surgery
Collection of Tissues, Cells, or Organs
Use of Biohazards or Chemical Agents
Use of Non-pharmaceutical-grade Chemicals or Other Substances
Use of DEA Controlled Substances
Breeding

Research Design

120.10 Research Design Instructions

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. Please attach such files as separate documents.*** *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.

120.11

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 the 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, or exposure to environmental factors that interfere with and/or enhance estrogen action increases 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 found during the follicular phase of the mother's menstrual cycle (i.e., <300 pg/ml). At the end of the first trimester, the placenta 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 levels exceed 5,000 pg/ml (fetal estrogen levels are about 20% of those in the mother). Based on our studies and proposed experiments, too much estrogen early in gestation (e.g., as can occur in "in vitro" pregnancies; exposure of mother to estrogen-likemolecules 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 the 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 the 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," which renders these vessels unable to respond to vasoactive agents and, thus, the vessels 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 "dumps" into and perfuses 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 the 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 of 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 the 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 will be mated with male baboons of proven fertility and pregnancy will be 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 post-puberty (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 post-puberty (n=8)

Study I Maternal Experiments (all groups): SEE ATTACHMENT
**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 programs tissues/organ systems of the fetus that impact insulin sensitivity, including skeletal muscle and including muscle structure and the number of micro vessels and, thus, the microvasculature. 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 and hypertension in adulthood. Our overall goal is to ascertain the mechanisms by which estrogen regulates development of the primate fetus 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 (e.g., the number of micro vessels, the number of slow/fast fibers, and expression of key regulatory factors in skeletal muscle; the number of pancreatic Beta cells). Acute in vivo studies are also performed to ascertain the mechanism of action of estrogen on angiogenic processes in fetal skeletal muscle that underpin formation of micro vessels. In vitro studies are also performed to determine the mechanism of estrogen action (e.g., 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 (e.g., cortisol, androgen serum levels), as well as glucose tolerance/insulin action and brachial artery flow (endothelial function) in presence/absence of vascular agents (e.g., acetylcholine) and skeletal muscle histology (number of micro vessels; number and size of slow/fast fibers; expression of growth factors; endothelial nitric oxide synthase) in muscle biopsies are studied prior to and after onset of puberty to determine the impact of estrogen programming of fetal organs (e.g., adrenal) and fetal metabolic systems on metabolic function in adulthood.

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 cesarean section (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)
Group 8: Maternal letrozole daily days 100-160; injection of estradiol into the fetus under ultrasound and delivery of fetus 6, 24, and 48 hrs later by c-section; one group of animals injected with vehicle control and delivered 48 hrs later.
NOTE: * ivGTT = intravenous glucose tolerance test; FMD = test flow mediated dilation of brachial artery

**Total number of pregnancies for Study II: N = 88 (8/group x 7 groups [n = 56] + 8 x 4 [n = 32] time frames for acute study)
**Total number of neonates delivered/reared for Study II: N = 24 (8/group x 3 groups)

Study II Maternal Experiments: SEE ATTACHMENT

PREGNANCIES (n=136) AND LIVE ANIMAL NUMBERS (n=156):
**Total number of pregnancies for Studies I and II: n = 136
**Total number of male breeders: n=9 (Pain Code Level B)
**Total number of adult females: n=35 (Pain Code Level D)
**Total number of spontaneous abortions for Studies I and II: n~9 (Pain Code Level B)
**Total number of fetuses for Studies I and II: n=63 (Pain Code Level C)
**Total number of neonates/offspring delivered/reared for Studies I and II: n = 40 (Pain Code Level D)

This research program continues to function as a collaborative effort with colleagues at the [redacted] as has occurred over the past 30 years. Thus, approximately 50% of the studies/animal treatments will be performed at the [redacted] and 50% will be performed at EVMS and tissue samples will be shipped between institutions. In addition, there are currently 20 offspring in our baboon colonies at EVMS and [redacted]. Therefore, a total of 58 pregnancies (136 pregnancies - 20 pregnancies with offspring already available = 116 needed pregnancies) are required at EVMS. As treatments are associated with a 20% loss due to spontaneous abortion or failure of neonates to thrive in an extrauterine environment, a total of 70 pregnancies (or 14 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 2 - 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 the investigative staff (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 'partially paired,' meaning they do not have continuous free interaction, but are restricted while allowing tactile contact and socialization on a limited level. Some animals cannot be successfully paired on 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 the absence of sexual skin swelling.

Blood Sampling:
Animals are sedated with ketamine (10-15mg/kg, intramuscularly (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. Total blood sample volume < 10% total blood volume not to exceed 10 ml/kg/month. Animals will be returned to their home cages and monitored for recovery.

Intravenous glucose tolerance test (ivGTT) at early and late gestation:
An ivGTT will be performed at early (~80-120d) and late (~140-160d) gestation. The baboon (14kg -20kg body weight) is fasted overnight and sedated with ketamine, intubated, and O2 delivered. The backs 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 is given. Once sedated, two baseline 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, 30, and 60 mins. During the experiment, the animal is constantly monitored for blood pressure (BP), heart rate (HR), and respiration, and it is warmed via a warming blanket. At completion of the experiment, the animal is given 1cc (10mg/kg) iron dextran, IM. Catheters are removed and the animal is monitored for swallowing reflex and response to stimuli before it is extubated. The animal is then returned to its cage.

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 baboons. Briefly, the baboon is fasted overnight and sedated with ketamine. The animal is 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 is given. Doppler flow analysis of brachial artery diameter and flow is determined before and after induction of shear stress. During the experiment, the animal is constantly monitored for BP, HR, and respiration, and it is warmed via a warming blanket. Baseline measurements of the brachial artery are taken once a 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 a stress response, a blood pressure cuff is placed distal to the brachial artery (wrist) and pressure is 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 its 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 the discussion of Study I, uterine artery remodeling is suppressed in baboons administered estradiol (25 µg/kg maternal body wt) daily on days 25-59 of gestation and it has been 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). The baboon is sedated with ketamine, a catheter is 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 is initiated. The animal is monitored for BP, HR, and respiration, and it is warmed via a warming blanket. A baseline blood sample (3-5ml) is obtained via an IV catheter to determine blood chemistries, gases and acid/base status, and subsequent analysis of estradiol, progesterone and androgens. The animal is infused with saline (0.5 ml/min) for 20 minutes, fetal heart rate is measured/monitoredm and uterine, umbilical, and fetal middle cerebral arterial blood flow dynamics are 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, fetal heart rate is continuously measured, and blood flow/ chemistry studies are 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 are 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 if fetal demise appears imminent, a cesarean section will be performed after consulting with the AV as described in this protocol. Fetal HR is checked at the start and finish of the experiment via ultrasound. At completion of the experiment, catheters are removed and the animal is monitored for swallowing reflex and response to stimuli before it is extubated. The animal is then returned to its cage.

Ultrasound guided fetal injection:
On day ~160-175 of gestation, the baboon is sedated with ketamine and placed on isoflurane gas. Ultrasound is performed to identify both the placenta and the fetus. Once localized, a 3-inch 23 gauge needle is inserted through the uterine wall into the rump or shoulder of the fetus and estradiol in 1.0ml saline/5% ethanol is injected. The needle is removed and the fetal HR is rechecked. The baboon mother is returned to its home cage and 6-24 hours later the fetus is delivered via c-section.

Cesarean section:
On days ~60 to ~170 of gestation based on the study group, the baboon is sedated with ketamine (10-15 mg/kg), intubated, and anesthetized with isoflurane/oxygen. Vitals (e.g., HR, BP, CO2, RR, and temperature) are monitored by CompMed staff. A

catheter is placed in the antecubital/brachial vein and IV fluids are administered. A second catheter is placed in the saphenous vein for blood sampling using a 19g catheter 24 inches in length and IV fluids are 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 a 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 4-24 cm in length depending on gestational age. Once the abdomen is open, the uterus is externalized and examined for placenta location. The 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. The 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 (surgery) forms located at the end of this document.

SURGERIES/PROCEDURES IN ALL EVMS-BORN NEONATES:

Blood Sampling:
Babies are examined daily by [redacted] 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, and weighed. A gross physical examination is performed by the PI staff and blood samples (3 ml) are obtained using a 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 their home cages 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, the baboon is fasted overnight and sedated with ketamine. The animal is intubated and O2 is delivered. The backs of both legs are shaved for catheter placement. Anesthesia is achieved via IV into an antecubital vein and a constant infusion is given of ketamine (0.1 mg/kg) and propofol (0.2 mg/kg) 0.6ml/minute. Once sedated, two baseline blood samples are collected and blood glucose and blood chemistries/gas levels are 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, and respiration, and it is warmed via a warming blanket. In the ivGTT performed at 24-30 months of age and in one ivGTT performed at a 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 the findings to insulin sensitivity/resistance as determined by the ivGTT. See Attachment E (surgery) for a complete biopsy description. At completion of the experiment, the animal is given 1cc (10mg/kg) of iron dextran, IM. Catheters are removed and the animal is monitored for swallowing reflex and response to stimuli before it is extubated. The animals is then returned to its home cage.

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 performed as outlined above. Once the second biopsy is taken, the animal will be euthanized via IV injection of Beuthansia-D solution. Once death is confirmed by the absence of a heart beat, 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, The baboons is fasted overnight and sedated with ketamine. The animal is intubated and O2 is delivered. Anesthesia is achieved via IV into an antecubital vein and a constant infusion id given of ketamine (0.1 mg/kg) and propofol (0.2 mg/kg) 0.6ml/minute. Doppler flow analysis of brachial artery diameter and flow are determined before and after induction of shear stress. During the experiment, the animal is constantly monitored for BP, HR, and respiration, and it is warmed via a warming blanket. Baseline measurements of the brachial artery are taken once a 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 is 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. 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 is monitored for swallowing reflex and response to stimuli, extubated, and returned to its 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 - Response to vasoactive agents:
Experiments are performed prior to puberty at ~24-30 months and repeated in the post-pubertal period at 5-14 years of age. Briefly, the baboon is fasted overnight and sedated with ketamine. The animals is intubated and O2 is delivered. Anesthesia is achieved via IV into an antecubital vein and a constant infusion is given 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, and respiration, and it is warmed via a warming blanket. Blood (3-5ml) is collected via the saphenous or femoral vein for analysis of blood chemistries and acid/base and gases (I STAT), and saline is infused (0.2 ml/min) via the antecubital vein. Doppler analysis of brachial artery diameter (right or left arm) and volume flow are then determined over a 5 minute period (~7 measurements). The baboon will then receive an infusion of pharmaceutical grade phenylephrine (1 and 5 µg/kg BW/min/0.3 ml saline) or pharmaceutical grade nitroprusside (1 and 3 µg/kg BW/min/0.3 ml saline) or 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 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 doses administered 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 the conclusion of the infusion of vasoactive agents, another blood sample (3-5 ml) will be obtained to verify blood chemistries and gases and acid/base status of the animal. 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 will be taken under the direction of the AV. At least one month later, the analyses are repeated, but phenylephrine (or nitroprusside or acetylcholine ± L-NAME) is 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 5-14 years of age). Once measurements are complete, anesthesia will stop and the animal is monitored for swallowing reflex and response to stimuli, extubated, and returned to its home cage to be monitored until upright. See Attachment E (surgery) for a complete biopsy description.

120.12 Attached files

Experimental Treatment Groups-b (3).docx Miscellaneous

Experimental Treatment Groups: Study I

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)

Experimental Treatment Groups: Study II

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)
Group 8: Maternal letrozole daily days 100-160; injection of estradiol into the fetus under ultrasound and delivery of fetus 6, 24 and 48 hrs later by cesarean section; one group of animals injected with vehicle control and delivered 48hr later.

Total number of pregnancies for Study II: N = 88 (8/group x 7 groups [n = 56]+ 8 x 4 [n = 32] time frames for acute study)

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 **
8	With Letrozole(~d100-d170)injection of estradiol to fetus rump/shoulder under ultrasound	2-4 day intervals under ketamine sedation				C-section (d160-170) 6, 24, or 48hr following injection -fetus euthanized

* ivGTT = intravenous glucose tolerance test; FMD = test flow mediated dilation of brachial artery]
** Studies in offspring described in surgery description

Overall total number of pregnancies for Studies I and II: N = 136
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 [redacted] as has occurred over the past 30 years. Thus, approximately 50% of the studies/animal treatments will be performed at the [redacted] and 50% at EVMS and tissue samples shipped between Institutions. In addition, there are currently 20 offspring in our baboon colonies at EVMS and [redacted] Therefore, a total of 58 pregnancies (136 pregnancies - 20 pregnancies with offspring already available = 116 needed 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 70 pregnancies (or 14 pregnancies/year over a 5-year period) are required to complete the objectives outlined.

120.20
Adverse Effects: Monitoring and Management:

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 the 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 the propofol dosage to maintain sedation, we will intubate the 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 in the event of an overdose. If GI upset is observed (loss of appetite), an alternate medication will be given in consultation with the Attending Veterinarian.

Isoflurane: Isoflurane will be inhaled to maintain a 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, isoflurane administration will be increased. Possible side effects can be hypotension, dose-dependent respiratory suppression, cardio depression, and GI effects (nausea, vomiting, ileus). If the animal shows a decreased heart rate, decreased blood pressure, or pale gum color with reduced capillary refill time (CRT), isoflurane administration will be decreased, along with a decreased intravenous fluid flow rate.

Ketoprofen: Oral (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 protocol.

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. The infection rate has been minimal to none in the 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): Possible complications may include bruising and discomfort at the site and infection at the site. Reduced movement may be observed for a short period of time. Analgesia is given to prevent discomfort.

IVGTT: Side effects are minimal. Short-term anemia and depreciated appetite from sedation are possible. Animals are given an Iron Dextran injection at completion of the experiment and supplemented with a children's vitamin containing iron. Also, food intake following experimentation 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 consequences. 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 are anticipated. A 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 will be consulted for treatment options.

Phenylephrine: Irregular heart rate, respiratory changes, allergic rash are possible. HR, BP, and body temperature 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 will be consulted for treatment options. At this low dose, we do not anticipate any problems or changes in animal behavior.

Acetylcholine: The agent is 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 if mean arterial BP drops below 40 mm Hg, the infusion will be stopped and the veterinarian will be consulted for treatment options.

N-nitro-L-arginine methyl ester (L-NAME): This drug blocks the vasodilatory effects of acetylcholine (i.e., blocks endothelial cell nitric oxide production) and, thus, could cause hypertension and a decrease in heart rate. However, this is unlikely at the dose employed. The drug has been used in human studies at doses 1.5 times greater than the dose proposed in this protocol without causing any significant change in BP or HR. The drug is not available in pharmaceutical grade. Chemical grade has been used in humans.

Serotonin: At the low dose proposed in this protocol, we do not anticipate any changes in animal behavior or any long-term

physiological 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 is suppressed, approximately 10% of the baboons will abort without any complications (visible vaginal bleeding). The products of conception may or may not be visible in the cage. In this case, the study will be terminated and the animal will be 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. In this case, the animals are typically found lying down (comatose) in their cages early in the morning, suggesting that the seizure(s) most likely occurred overnight or very early that morning. In animals that have seized, we believe it is important to intervene at the 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 has been used successfully in the past:
• Animals which are stuporous (unsteady on their feet but conscious) will immediately be given oral juice/sugar treatment in the form of frozen juice or a piece of orange or candy to elevate blood glucose levels. If the animal is non-responsive or progresses to seizing or to an unconscious state, the following regimen 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 (pO2, pC02, pH, etc.) and glucose will be determined using I-Stat analyzer (results in 2 mins).
• The animal will be placed on O2 at 2L/min via a cone mask and body temperature will be recorded and maintained with a 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 (i.e., glucose is 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 will be delivered by cesarean section at the end of treatment. It is important to note that of the 10% of animals 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 of 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 seizures will occur. CompMed personnel, as well as the PI staff, are aware of this possible linkage and are attempting to resolve this problem going forward. We do want to point out that regardless of when an animal seizes, we will employ the protocol outlined above. In addition, decreased appetite can be seen during late treatment with Letrozole. The animal's gums can become swollen, making hard biscuit consumption difficult. In this case, the affected animals will be given softened biscuits soaked in Ensure or Boost to assist in palatability and to ensure necessary nourishment. If an animal continues to exhibit inappetence despite this treatment or if it 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 are anticipated. Irritation of the 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 the 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 the animal's health is not compromised, blood collection will not exceed 10% of the circulating blood volume or 10ml/kg/month.

120.21 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 the PI staff and twice daily by the 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 the PI staff, with these observations recorded on postoperative evaluation sheets, which become part of each animal's permanent records. Postoperative evaluation will include specific assessment of pain, to include failure to eat or decreased appetite/drink, urination or fecal output, and change in normal repertoire of behaviors. Lethargy and guarding of the incision site(s) may also indicate pain. If any of the above-referenced conditions are seen during the postoperative monitoring period, the veterinarian will be notified. Animal weight is monitored weekly while the animal is on study when sedated. Blood chemistry are performed monthly as well. Any changes are relayed to the veterinarian and the recommended treatment regimen will be followed.

120.22 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 in accordance with the IACUC policy 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.

Administration of Anesthesia, Therapeutics, and Experimental Agents

130.10 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.

If you are unsure whether an agent is DEA regulated, please click here.

UNAUTHORIZED USE OF A DEA REGULATED SUBSTANCE MAY RESULT IN SUSPENSION OF THE IACUC-APPROVED PROTOCOL.

130.11 Does this project involve the use of one or more DEA regulated controlled substances?
Yes

130.12 Name of the DEA registrant.
[redacted]
Email: [redacted] **Assistant:** (757) [redacted]

130.13 DEA Research License Registration Number or Application Confirmation Date
[redacted]

130.14 DEA License Effective Date
10/27/2020

130.15 DEA License Expiration Date
08/31/2023

130.16

Name of controlled substance: Ketamine - HCl
DEA Schedule #: 3
Intended use: Sedation, restraint, anesthesia for procedures in the protocol
Dosage: 0.1mg/kg IV to 10-15mg/kg IM

Name of controlled substance: B-euthanasia-D solution
DEA Schedule #: 3
Intended use: Euthanasia
Dosage: 1mg/kg

130.20 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. Justification for withholding analgesic agents must be based upon cited scientific fact or provided experimental data.

130.21

: Analgesic
Agent name: Flunixin meglumine (Banamine)
Dose in mg/kg: 2mg/kg

Route
: IM
Frequency of administration: At surgery and muscle biopsies
Length of administration: Surgery and 2 days postop BID

: Anesthetic: General
Agent name: Ketofol
Dose in mg/kg: Ketamine 0.1mg/kg : Propofol 0.2mg/kg

Route
: IV infusion
Frequency of administration: IVGTT with/without biopsy, Flow Mediated Doppler (FMD)
Length of administration: 1-2 hours

: Anesthetic: General
Agent name: Isoflurane
Dose in mg/kg: to effect (~1-3% in 100% oxygen for maintenance)

Route
: Inhalation
Frequency of administration: C-section surgery and fetal injection
Length of administration: For the length of the procedure

: Anesthetic: General
Agent name: Ketamine
Dose in mg/kg: 10-15mg/kg

Route
: IM
Frequency of administration: sedation for all protocol procedures
Length of administration: Initial sedation

: Analgesic
Agent name: Ketoprofen
Dose in mg/kg: 75mg

Route
: PO
Frequency of administration: 2 days postop/muscle biopsy
Length of administration: Alternative to IM Flunixin injection when use of Ketoprofen is more suitable for a particular animal

: Miscellaneous agent
Agent name: Beuthanasia - D solution
Dose in mg/kg: 1mg/kg

Route

: IV

Frequency of administration: Once
Length of administration: Terminal procedure

: Miscellaneous agent
Agent name: Iron Dextran
Dose in mg/kg: 10mg/kg

Route

: IM

Frequency of administration: Following SX and ivGTT
Length of administration: Once

: Miscellaneous agent
Agent name: Terbutaline
Dose in mg/kg: 0.25mg

Route

: IV or SQ

Frequency of administration: PRN under vet consultation in response to fetal HR depression during the FMD procedure
Length of administration: PRN; to be repeated 30 mins if no clincial change occurs

130.22 If withholding analgesic agents, please give justification
N/A

130.30 Will agents other than anesthetics or analgesics (e.g., drugs, reagents, cells, etc.) be administered?

Yes

130.31

Agent: Letrozole

Agent Vehicle: Sesame Oil

Volume per administration: 0.2-2.0ml

Route: SQ

Site: Abdomen or back

Frequency of administration: daily during pregnancy

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 consequences of drug therapy are a decrease in estrogen production levels by >95%, we observe premature delivery and/or maternal seizures in 15-20% of pregnancies. In instances where a mild seizure occurs but the animal has not become comatose, we stop drug treatment for 24-48 hrs and monitor the animal. Drug treatment can resume without further development of problems.

Agent: Estradiol 17B-3 benzoate

Agent Vehicle: Sesame Oil; saline/5% ethanol

Volume per administration: 0.2-2.0ml

Route: SQ or IM to fetus

Site: Abdomen or back; rump or shoulder of fetus

Frequency of administration: Daily on ~ days 100-170 of gestation in conjunction with letrozole to restore estrogen production; also administered to the fetus on ~ day 160, IM

List all expected side effects and/or changes in the animal's behavior:: None are anticipated

Agent: Phenylephrine

Agent Vehicle: 0.9% saline

Volume per administration: 1-2ug/kg BW/min/0.3ml saline and then 5ug/kg/BW/0.3ml saline (each for 20minutes in step-up fashion

Route: IV

Site: 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, no side effects are expected

Agent: Acetylcholine

Agent Vehicle: 0.9% saline

Volume per administration: 4ug/kg/BW/0.3ml saline and then 8ug/kg/BW (each for 20min in a step up fashion)

Route: IV

Site: Brachial or Saphenous 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, no side effects are expected

Agent: Serotonin (5-HT)

Agent Vehicle: 0.9% saline

Volume per administration: 4ug/kg/BW/0.3ml saline and then 8ug/kg/BW (each for 20min in a step-up fashion)

Route: IV

Site: Brachial or Saphenous vein

Frequency of administration: Once per pregnancy

List all expected side effects and/or changes in the animal's behavior:: At this low dose, no side effects are expected

Agent: N-nitro-L-arginine methyl ester (L-NAME)

Agent Vehicle: 0.9% saline

Volume per administration: 40ug/kg bw/min

Route: IV

Site: Brachial or Saphenous 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 HR; however, this is unlikely at this dose. Pharmaceutical grade is not available and chemical grade has been used in humans.

Agent: Dextrose solution (50%)

Agent Vehicle: In solution

Volume per administration: 0.25grams/kg BW

Route: IV

Site: Brachial or Saphenous vein

Frequency of administration: once at IVGTT

List all expected side effects and/or changes in the animal's behavior:: Local pain and irritation at the injection site are possible. Diabetic coma, delirium tremors, and congested states are possible, but unlikely. HR and BP are measured before and after injection.

130.32 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-NAME, is not available in pharmaceutical grade and chemical grade has been used in humans. Acetylcholine, estradiol-17ß-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) and are already dosed and in liquid to be used as an eye drop or inhalant (acetylcholine), as an 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 such as estradiol). Chemical grade reagents are available and product information indicates preparations are very high grade (>99% pure). Finally, we have used and were previously approved under IACUC #18-006 to use chemical grade phenylephrine, serotonin, estradiol17ß-3 benzoate, and acetylcholine in our pregnant baboons and/or their offspring with no apparent untoward or harmful effects.

130.40 NOTE: Your signature on this form 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. <u>All controlled substances MUST be kept in a double-locked compartment, and records documenting each use of a controlled substance MUST be maintained.</u>

Species selection and ordering

140.10 Species Selection and Ordering

140.11 Please indicate the species, strain, and number of animals requested in the table below. Please click save after every row.

Species name and strain	Total requested for 3 year period	Average # to be maintained in the animal facility	Maximum # to be maintained in the animal facility
Baboon (Papio anubis/cynocephalus)	156	35	50

140.12 Will animals be ordered through the Division of Comparative Medicine (CompMed)?
Yes

140.14 Will special housing be required (e.g., specific bedding requirements, isolator cages, special feed or handling, etc.)?
Yes

140.15 Describe all special requirements.
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 housed individually in double cages (two cages joined together) and females are housed individually in a single cage. A male baboon is pair housed for 2-5 days with a female during her period of estrous for the purposes of mating, thus, for breeding purposes, the male and female share a double cage (two cages joined together). This increases mating contact and has proven to yield higher pregnancy rates with no increase in incidences of altercations. Also, we have had no adverse injuries from this breeding design. Importantly, animals are not stressed as females do become pregnant (e.g., ovulate and mate) and males produce sperm and mate.

Some adult females are incompatible with others and physical trauma arises as evidenced by over-grooming, nutritional issues (an animal loses weight from not eating), or over-submissive behavior. Other reasons for singly housing animals are self-injurious behavior (SIB), which can be stressful to a cage mate. A late gestation pregnant animal expected to deliver spontaneously will be singly housed during late gestation to ensure safe delivery of the fetus.

At a minimum, most animals are partially paired, which allows liberal touch, but full range contact may be limited.

Semi-annual re-evaluation of the colony attempts to reintegrate the singly housed animals. At a minimum, all animals will have a visual, auditory, and olfactory sense of other NHPs.

NOTE: 3-5 adult baboons/year will be purchased to maintain the colony.

140.16 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.")
Yes

140.17 List the number of new animals to be purchased for the colony:
5

140.18 List the number of animals to be used from an existing colony:
22

Personnel Training

150.10 Please fill out the information for all personnel involved with the project and enter all functional roles they have. Please click SAVE at the top right of each card AFTER filling into the information fully.

Name: [redacted]
Species: Non-Human Primate
Years of experience with that species: 45
OHSP certification number: [redacted]
OHSP test expiration date: 03/27/2023
OHSP NHP test expiration date: 08/27/2023
Occupational health risk assessment expiration date: 07/07/2021
CITI certification number: [redacted]
CITI Base Course: 02/12/2006
CITI Base Refresher Course (3-year expiration): 04/07/2024
CITI NHP: 08/17/2022
CITI Aseptic Surgery: Missing
Functional role: Supervision
Care and Handling
Surgery
Monitoring
Euthanasia

Name: [redacted]
Species: Non-Human Primate
Years of experience with that species: 20
OHSP certification number: [redacted]
OHSP test expiration date: 03/11/2023
OHSP NHP test expiration date: 08/27/2023
Occupational health risk assessment expiration date: 09/18/2021
CITI certification number: [redacted]
CITI Base Course: 06/03/2005
CITI Base Refresher Course (3-year expiration): 03/17/2024
CITI NHP: 08/27/2022
CITI Aseptic Surgery: 01/01/2099
Functional role: Supervision
Care and Handling
Anesthesia
Surgery
Post-Surgical Care
Monitoring
Euthanasia

Name: [redacted]
Species: Non-Human Primate
Years of experience with that species: 20
OHSP certification number: [redacted]
OHSP test expiration date: 03/13/2023
OHSP NHP test expiration date: 09/02/2023
Occupational health risk assessment expiration date: 07/19/2021
CITI certification number: [redacted]
CITI Base Course: 01/30/2006
CITI Base Refresher Course (3-year expiration): 02/29/2024
CITI NHP: 02/05/2023
CITI Aseptic Surgery: Missing
Functional role: Care and Handling
Surgery

Monitoring

150.11 Provide information regarding the degree of training and procedural experience for each individual listed as personnel on this protocol.

[redacted] has ~45 years of experience working with NHPs and he 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 as in providing surgical assistance. Moreover, [redacted] has been trained by [redacted] and has been performing surgeries (cesarean section) for 20 years without the direct assistance of [redacted] Although [redacted] does not scrub in for surgeries, he is available and will routinely be on-site to assist in the collection of fetal tissues. [redacted] in consultation with [redacted], actually performs all of the animal husbandry (e.g., animal injections, blood sampling, etc.) and she works with the CompMed staff and other laboratory personnel in hand-rearing of baboon neonates per the approved SOP.

[redacted] has had significant experience assisting [redacted] in the conduct of the surgical procedures and in collecting tissue samples. She will perform many of the biochemical analyses outlined in the protocol and she will assist in the preparation of fixative and all reagents used in these studies, with the exception of Letrozole and analgesics. [redacted] will assist in collection of and performing biochemical analyses of baboon fetal tissues and biopsies of skeletal muscle from adolescent baboons. She will also assist [redacted] with conducting and analyzing the IV glucose tolerance tests and brachial artery flow mediated (FMD) studies and with conducting ultrasound studies of uteroplacental/fetal blood flow.

150.12 List any person who will require supplemental training from CompMed and state the training required for each person.

N/A

Animal Procedures

160.10

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

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 analgesiap re- 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 infection;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 (smallburr hole),Placement of vascular access ports;Orchiectomy;Superficial lymphnode 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

160.20 Animal Use Procedures (EXCEPT SURGICAL PROCEDURES)

160.21 Will cells, tissues, and/or organs be collected?

Yes

160.30 If collecting blood samples, please list technique used, sample site(s), volume per sample, and frequency/duration of sampling.

[1] Pregnancy studies: Blood samples (maternal) are collected at 2-4 day intervals depending on the study group. Briefly, animals are restrained and injected with ketamine-HCl (10-15 mg/kg) and samples (3-5 ml) are 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 and injected with ketamine HCl (10-15 mg/kg BW) and a 3 ml (neonates) sample is 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 in syringes via a catheter at 00, 0, 1, 3, 5, 10, 20, 30, 60 and 90 min. 0.1 ml is examined for blood glucose and the remainder is kept on ice. Serum is subsequently assayed for insulin/C-peptide.

[4] Doppler Studies: Two samples are taken (1 = start of the experiment and 1 = completion of experiment) for blood chemistry and hormone analysis.

160.40 If collecting urine or fecal samples, please list sampling method, and frequency/duration of sampling.

N/A

160.50 If collecting tissue samples, please list all tissues to be collected, when the tissues will be collected (specifically before or after euthanasia), and final disposition of the collected tissues.

Tissues to be collected: 1) Kidney, liver, lung, gonads, adrenal, pitultary, pancreas, skeletal muscle, visceral and SQ fat, intestine, heart and uterine samples following euthanasia; and 2) muscle biopsy with or without IVGTT at time 0 and 30 min.

Final disposition of the collected tissues: Fixed and/or frozen for experimentation (histopathology)

160.60 Will Behavioral Testing be conducted?

No behavioral testing will be conducted.

160.70 Will a special diet be required?

No

160.80 Will indwelling catheters or implants be used?

No

160.90 Will tumors be transplanted or induced?

No

Animal Care

170.10 Identify all investigative staff responsible for providing animal care

[redacted]

NOTE: Routine observation of the animals and medical intervention is the responsibility of the Principal Investigator.

170.11 Describe in detail the plan for medical care of the animals in the proposed study.
All animals will be observed daily by [redacted], Animal Coordinator/Research Associate, and/or the 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 in compliance with 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 in Section 120.21. Postoperative observations are recorded on forms approved by CompMed, and these forms become part of each animal's permanent record.

170.20 Will a special observation regimen be required?

Yes

170.21 Frequency of observation
Daily once on study; seven days post-operatively; three days following muscle biopsy

170.22 Who will do the special observation?
[redacted]

170.23 Starting:
Start of study/pregnancy

170.24 Ending:
Post-operatively to the end of the study/euthanasia

170.30 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 the uterus and adrenals, and pieces of the kidney and liver (or other organs/tissues as determined by the veterinarian), which are placed in fixative (4% paraformaldehyde or phosphate buffered formalin) for subsequent histopathology.

Disposition of Animals

180.10 Please indicate the method of animal disposition. Check all that apply.

Euthanasia
Return to the animal colony
Available for transfer to another EVMS IACUC-approved protocol
Available for transfer to another research institution

180.11 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.

180.20 Will animals survive the protocol procedures? If some animals will survive and others will not, please indicate both YES and NO.

Yes
No

180.21 Please clearly state which animals will survive, and which animals will not survive.
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 for health reasons, all under consultation with the veterinarian.

180.30 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.

Yes

180.40 Will the animals be euthanized?

Yes

180.41 Explain why the animals will be euthanized.
The maximum number of multiple survival surgeries has been achieved, the animal has developed protocol-related or clinical problems, 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 a need to collect adult tissues (e.g., ovaries, adrenals, 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 animals (<5%) be transferred to the [redacted] to be used in our study there.

180.42 Indicate how the animals will be euthanized. Include euthanasia agent/procedure, dose of administration, and route of administration.
IV injection of Beuthanasia-D solution (1mg/kg) at the time of the terminal procedure

180.43 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

Animals Brought into and Taken Outside of the Animal Facility

190.10 Will animals be transferred into the CompMed animal facility from another institution?

Yes

190.11 Name and location of the transferring institution:

[redacted]

190.12

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.

190.13 How long will the quarantine or stabilization period last?

Until completion of 3 negative TB tests or as determined by the Attending Veterinarian

190.14 How long will the animals be housed at EVMS?

3+ years until completion of the study

190.20 Will the animals be taken out of the CompMed central animal facility for any reason (e.g., manipulation, surgery, temporary housing, etc.)?

No

190.30 Will the animals be used or housed in locations outside of the CompMed central animal facility for more than 12 hours?

No

Attachement B: NHP Enrichment

191.10 Attachment B Instructions

Current federal regulations require that facilities develop, document and follow a plan for promoting the psychological wellbeing of captive nonhuman primates (9 CFR, Subchapter A). The plan must address the social needs of the primates housed in a facility and provide some form of environmental enrichment to promote species typical behavior. The EVMS "Nonhuman Primate Enhancement Program" amended and approved by the IACUC in June, 2000 follows the recommendations of the Committee on Well-Being of Nonhuman Primates, Institute of Laboratory Animal Research, National Research Council book The Psychological Well-Being of Nonhuman Primates, National Academy Press, 1998. The Division of Comparative Medicine (CompMed) provides enrichment, but investigators are encouraged to, and commonly do, participate in the program. To achieve the goals of a well-designed plan the following aspects must be addressed:

- *Appropriate social companionship*
- *Opportunities to engage in behavior related to foraging, exploration and other activities appropriate to the species, age, sex, and condition of the animals*
- *Housing that permits suitable postural and locomotor expression*
- *Interactions with personnel that are generally positive and not a source of unnecessary stress*
- *Freedom from unnecessary pain and distress*

Taken from The Psychological Well-Being Of Nonhuman Primates

Social Enrichment

A. *Pair housing and group-housing is considered for all primates based upon species, age, history and nature of the primate. Special consideration for young animals is required.*

B. *Social interactions are considered one of the most important factors influencing the psychological well being of most nonhuman primates. The justification for single housing must be adequately described in the protocol and approved by the IACUC.*

Individual Enrichment

A. *Housing: Primates are housed in environmentally controlled rooms providing for visual, auditory, and olfactory interaction. Enrichment of the home cage environment is accomplished with a variety of devices and materials in addition to the basic standards of cage design. These devices are designed to address foraging (puzzle feeders, wood shavings) and play or curiosity (kong toys, balls, pipes, chew toys, etc.) needs of the animals.*

B. *Food: The core of the diet consists of standard commercial monkey chows. An appropriate amount is given to the monkey through conventional means. The standard diet is regularly supplemented with fruits and vegetables (three times a week). Further supplements include treats and puzzle-feeders administered during human interactive periods.*

C. *Human Interaction: The DAR employs an enrichment coordinator full time with training and interest in nonhuman primate behavior. In addition, EVMS has a Behavioral Consultant on advisement for any questions that may arise in the implementation of the plan. Animal care technicians and research staff are actively recruited to participate in the enrichment opportunities of human/animal interaction. While performing daily husbandry duties and research protocols, the animal care technicians become familiar with behavior patterns and are able to identify individual needs and deliver prescribed regimens. All aberrant behaviors are reported to the institutional veterinarian for evaluation and treatment.*

Monitoring of "Psychological Well-Being"

Primate Evaluation Criteria:

- *The daily assessment of health (activity, appetite and physical signs).*
- *The daily assessment of behavioral state. Including knowledge of species typical behavior.*
- *Record keeping documenting the presence, etiology, and remediation or accommodation of observed cases of lack of well being.*

Exemption Criteria

Non-Human Primates may be exempted from participating in some of the otherwise required environmental enhancement plans on an individual basis. The USDA Animal and Plant Health Inspection Service allows only the following exemption procedures:

IACUC*: An individual animal may be exempted for scientific reasons by the IACUC. The basis of the exemption is documented in the approved protocol and is reviewed at appropriate intervals determined by the IACUC, but not less than annually.*

Veterinarian*: An individual animal may be exempted because of its health, aggression or condition (or in consideration of its overall well being by the attending veterinarian). The basis of the exemption is recorded by the attending veterinarian for each exempted animal. Unless the basis for the exemption is a permanent condition, the attending veterinarian must review the exemption every 30 days.*

191.11 Paired Housing
Yes

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

191.13 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).
Yes

Attachment D: Use of Hazardous Agents (1 of 3)

193.0.10

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. IF YOU HAVE MORE THAN ONE AGENT, PLEASE CLICK REPEAT AT THE END OF THE FORM TO ADD ANOTHER AGENT AS NEEDED.

193.0.11 Please indicate the type of hazardous agent being used.
Known or suspected human mutagen/teratogen

193.0.17 chemical name
Letrozole

193.0.20 Please provide the complete name of the agent, including strain for microorganisms

Letrozole

193.0.21 Dose and frequency of administration
0.2 to 2.0ml/kg BW/day

193.0.22 Concentration
2mg/ml

193.0.23 Route:
SQ

193.0.24 Duration of exposure
10-70 days depending upon the study

193.0.25 How long will the animal be maintained after administration?
Animals that survive the study will be maintained until they reach the allowable 6 survival surgeries. If complications are determined by evaluation of the AV, the animal will be terminated upon completion of the study; however, this has not been a common occurrence and is not expected.

193.0.26
Estimated animal weight

14-20 kg

193.0.30 Is the agent excreted or shed by the animal?

No

193.0.40 Are there documented human risks from exposure to the agent?

Yes

Risks may be determined from the MSDS or from references found in the Biosafety Manual.

193.0.41 Indicate the route of human exposure. Click all that apply
Inhalation
Ingestion
Contact

193.0.43 Describe the risks associated with the agent (mutagen, teratogen, etc.):
The agent is known to suppress estrogen production in females. Letrozole is an oral, anti-estrogen drug used for treating postmenopausal women with breast cancer.

193.0.44 Describe any genetic changes to the organism and their suspected effects:
N/A

193.0.45 Indicate the acutely toxic/infectious dose in humans (in mg/kg or number of organisms)
The standard dose of Letrozole used in women is 2.5mg/day. We will be administering a maximum of 2mg/day and do not anticipate any acute side effects of the drug per se. Only PI staff is exposed to the agent.

193.0.46 Describe the symptoms of exposure
Exposure would have to be long-term and the agent needs to be ingested or there needs to be contact with an open wound. Basic exposure symptoms would be irritation or a skin rash. We have not seen any exposure symptoms using Letrozole over the past 25 years.

193.0.47 Describe the first aid methods to be taken in the case of exposure:
Flush the area with water

193.0.48 Indicate all personal protection required. Check all that apply.
Lab coat/dedicated clothing
Gloves
Goggles
Other

193.0.49 Please describe
Mask

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

No

193.0.60 Are special waste or carcass disposal requirements necessary?

No

193.0.70 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.

The drug is prepared at a concentration of 2mg/ml in sesame oil. Animals are injected with 0.2-2.0ml subcutaneously. The drug is not shed or excreted by the animals.

193.0.80 Please indicate whether or not the laboratory personnel have been informed of these hazards and have received training on proper handling techniques.

Yes

193.0.90 Please indicate whether or not the CompMed staff have been informed of these hazards and have received training on proper handling techniques.

Yes

193.0.110 Please provide any other relevant information regarding the agent (e.g., unique containment recommendations):

N/A

193.0.120 Please attach any other documents that would be relevant to this material, such as SDS sheets, manufacturer information, etc.

No answer provided.

193.0.10

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.***

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YOU MUST COMPLETE A SEPARATE FORM FOR EACH HAZARDOUS AGENT USED. IF YOU HAVE MORE THAN ONE AGENT, PLEASE CLICK REPEAT AT THE END OF THE FORM TO ADD ANOTHER AGENT AS NEEDED.

193.0.11 Please indicate the type of hazardous agent being used.
Acutely toxic chemical

193.0.14 CAS#
59-42-7

193.0.15 LD50:
in mice 120mg/kg; in rats 30mg/kg

193.0.20 Please provide the complete name of the agent, including strain for microorganisms

Phenylephrine

193.0.21 Dose and frequency of administration
1-5ug/kg for 10-30 minutes (20 min on average) in a step-up fashion

193.0.22 Concentration
stock solution 1mg/ml

193.0.23 Route:
IV infusion

193.0.24 Duration of exposure
no more than 30 minutes

193.0.25 How long will the animal be maintained after administration?
The animal will be returned to its home cage and used in other study groups, since no long-term effects are expected.

193.0.26
Estimated animal weight

4-20kg

193.0.30 Is the agent excreted or shed by the animal?

No

193.0.40 Are there documented human risks from exposure to the agent?

Yes

Risks may be determined from the MSDS or from references found in the Biosafety Manual.

193.0.41 Indicate the route of human exposure. Click all that apply
Inhalation
Ingestion
Contact

193.0.43 Describe the risks associated with the agent (mutagen, teratogen, etc.):
The agent can cause a sudden rapid increase in blood pressure; however, most overdoses require only patient observation as the material has a very short duration of action and removing the agent will return the animal to normal conditions. All animals are monitored for HR and BP during infusion studies

193.0.44 Describe any genetic changes to the organism and their suspected effects:
N/A

193.0.45 Indicate the acutely toxic/infectious dose in humans (in mg/kg or number of organisms)
No actual dose is found. LD50 in mice is 120mg/kg and in rats 350mg/kg

193.0.46 Describe the symptoms of exposure
Exposure would have to long-term and contact would have to be ingestion or contact to an open wound. Basic exposure would be skin irritation or rash.

193.0.47 Describe the first aid methods to be taken in the case of exposure:
Flush the area with water

193.0.48 Indicate all personal protection required. Check all that apply.
Lab coat/dedicated clothing
Gloves
Goggles
Other

193.0.49 Please describe
mask

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

No

193.0.60 Are special waste or carcass disposal requirements necessary?

No

193.0.70 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.

The agent is administered IV over a 30min period. The active agent is metabolized by the liver and excreted by the kidneys. Concentration is minimal and undetectable in excretion.

193.0.80 Please indicate whether or not the laboratory personnel have been informed of these hazards and have received training on proper handling techniques.

Yes

193.0.90 Please indicate whether or not the CompMed staff have been informed of these hazards and have received training on proper handling techniques.

Yes

193.0.110 Please provide any other relevant information regarding the agent (e.g., unique containment recommendations):

N/A

193.0.120 Please attach any other documents that would be relevant to this material, such as SDS sheets, manufacturer information, etc.

No answer provided.

193.0.10

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. IF YOU HAVE MORE THAN ONE AGENT, PLEASE CLICK REPEAT AT THE END OF THE FORM TO ADD ANOTHER AGENT AS NEEDED.

193.0.11 Please indicate the type of hazardous agent being used.
Known or suspected human mutagen/teratogen

193.0.17 chemical name
Estradiol-17B 3 Benzoate

193.0.20 Please provide the complete name of the agent, including strain for microorganisms

Estradiol-17B 3 Benzoate

193.0.21 Dose and frequency of administration
25ug/kg (SQ, abdomen or back of the adult female) for 35 days in early gestation of pregnancy or 115ug/kg for 70 days in late gestation of pregnancy; the agent will also be administered to the fetus (IM, rump or shoulder) on ~ day 160 of gestation

193.0.22 Concentration
2mg/ml

193.0.23 Route:
Subcutaneous (SQ; adult female)
Intramuscular (IM; fetus)

193.0.24 Duration of exposure
35-70 days determined by the study

193.0.25 How long will the animal be maintained after administration?
The animal will be returned to its home cage for subsequent studies, since no long-term effects are expected.

193.0.26
Estimated animal weight

14-20kg

193.0.30 Is the agent excreted or shed by the animal?

No

193.0.40 Are there documented human risks from exposure to the agent?

Yes

Risks may be determined from the MSDS or from references found in the Biosafety Manual.

193.0.41 Indicate the route of human exposure. Click all that apply
Inhalation
Ingestion
Contact

193.0.43 Describe the risks associated with the agent (mutagen, teratogen, etc.):
Cancer of the female reproductive tract is often due to exposure to endogenous estradiol and, thus, chronic exposure to estradiol 3-benzoate is likely also associated with increased risk for cancer.

193.0.44 Describe any genetic changes to the organism and their suspected effects:
N/A

193.0.45 Indicate the acutely toxic/infectious dose in humans (in mg/kg or number of organisms)
Chronic estradiol exposure can induce menstrual irregularities and elicit estrogenic effects in males (e.g., breast enlargement). Estrogen is potent at low doses (ug/kg BW) but is rapidly metabolized (half life of 90 min). Only the PI staff is exposed to the agent.

193.0.46 Describe the symptoms of exposure
Exposure would have to be long-term and the agent need to be ingested or there needs to be contact with an open wound. Basic exposure symptoms would be skin irritation and a rash.

193.0.47 Describe the first aid methods to be taken in the case of exposure:
Flush the area with water.

193.0.48 Indicate all personal protection required. Check all that apply.
Lab coat/dedicated clothing
Gloves
Goggles
Other

193.0.49 Please describe
Mask

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

No

193.0.60 Are special waste or carcass disposal requirements necessary?

No

193.0.70 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.

Daily subcutaneous injection administered to pregnant baboons for 35-70 days; the agent is first converted to the active hormone, estradiol 17B (benzoate removed), and then catabolized to estrone and glucuronylated and excreted via the kidneys/urine or via stool

193.0.80 Please indicate whether or not the laboratory personnel have been informed of these hazards and have received training on proper handling techniques.

Yes

193.0.90 Please indicate whether or not the CompMed staff have been informed of these hazards and have received training on proper handling techniques.

Yes

193.0.110 Please provide any other relevant information regarding the agent (e.g., unique containment recommendations):

N/A

193.0.120 Please attach any other documents that would be relevant to this material, such as SDS sheets, manufacturer information, etc.

No answer provided.

Attachment E: Animal Surgery

194.0.10 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.

194.0.20 Pre-Operative Procedure

194.0.21 List all persons responsible for evaluating the health status of animals
[redacted].

194.0.22 Will food be withheld?
Yes

194.0.23 Explain why food will be withheld and state how long it will be withheld.
Animals will be fasted overnight prior to all procedures to reduce the chance of vomiting and the potential for aspiration during the procedures.

194.0.24 List all pre-operative anesthetic and/or analgesic agents to be used (i.e., name and dosage for each agent).
Initial sedation will be with Ketamine (10-15mg/kg), IM

194.0.25 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. Vitals (e.g., HR, BP, CO2, RR, and temperature) are monitored by the CompMed staff. A catheter is placed in the antecubital/brachial vein and IV fluids are administered. A second catheter is placed in the saphenous vein for blood sampling using a 19g catheter 24 inches in length and IV fluids are 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.

194.0.30 Anesthetic Procedure

194.0.31 Will the animals be anesthetized?
Yes

194.0.33 Please list all persons who will administer the anesthesia
[redacted]

194.0.34 List the anesthetic agent(s) to be used (i.e., name and dosage) and the route of administration for each agent.
Isoflurane gas is vaporized with MAC of 1-3% in 100% oxygen via an intubation tube. Anesthesia monitoring sheets are maintained by the CompMed staff and stored in the animal's record.

194.0.35 List all persons who will keep anesthesia records
[redacted]

194.0.36 Explain how anesthetic recovery will be monitored.
At completion of the experiment, the animal is given 1cc (10mg/kg) of iron dextran, IM. Catheters are removed and the animal is monitored for swallowing reflex and response to stimuli before it is extubated. Vitals are monitored until the animal is extubated. The animal is then returned to its cage.

The CompMed staff is primarily responsible for immediate anesthesia monitoring. [redacted] will also be present.

194.0.37 List all persons who will monitor the recovery.
[redacted]

194.0.40
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.

194.0.41 List all persons who will monitor daily post-operative care.
[redacted]

194.0.42 List all locations where post-operative records will be kept.
Post-op sheets will be kept outside the animal room until the procedure is completed, after which they will be placed in the animal's medical record maintained in the CompMed office.

194.0.43 List all persons who will keep post-operative records.
[redacted]

194.0.44 Will post-operative analgesics be administered?
Yes

194.0.46 Provide the following information for each post-operative analgesic agent to be administered. Please click save after inputting the information for each agent.

Agent: Flunixin meglumine (Banamine)
Dose: 2mg/kg
Route: IM
Frequency: post procedure and 2 days post-operatively BID
Post operative Duration of care: 5-7 days (BID for 3 days PRN for remainder of the procedure)

Agent: Ketoprofen
Dose: 75mg
Route: PO
Frequency: 2 days post-operatively
Post operative Duration of care: 5-7 days as an alternative to Flunixin

194.0.50
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

194.0.51 Will the animals be subjected to more than one survival surgery?
Yes

194.0.52 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) and 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 to be performed is a cesarean section.

194.0.53 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 of procedures 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 (e.g., excessive adhesions, uterine windows, endometriosis, etc.) that would not be compatible with performing further surgeries.

194.0.54 Have any animals to undergo major survival surgery in this protocol already undergone major survival surgery in any other IACUC protocol(s)?
Yes

194.0.55 Identify all animals that have undergone prior surgical procedures in another protocol.
Under IACUC #18-006:
*28768 has had 3 survival surgeries
*26876 has had 2 survival surgeries
*27320 has had 1 survival surgery
*26741 has had 3 survival surgeries

Animals were not used in an unrelated protocol. This protocol is the 3-year continuation of IACUC #18-006.

194.0.56 Identify all previous procedures performed on these animals.
C-section survival surgeries

194.0.57 List the IACUC protocol number(s) under which the previous procedures were performed.
IACUC #18-006; this protocol is the renewal of IACUC #18-006

194.0.58 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 (the research is continuous). 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 mimic the situation in humans.

194.0.60 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

194.0.61 Classify each surgical procedure to be performed according to the table listing above.

Surgery Type	Procedure to be Performed
III	Cesarean section

(Example: Surgery Type: 1; Procedure to be performed: Bone marrow aspiration.)

195.0.10 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. CLICK REPEAT AT THE BOTTOM OF THE PAGE WHEN DONE FILLING INFORMATION ON ONE SURGICAL PROCEDURE TO SAVE AND OPEN A NEW PAGE FOR ANOTHER PROCEDURE AS NEEDED.

195.0.11 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

195.0.12 Is the procedure survival surgery?
Yes

195.0.13 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.
YES and NO:

YES - 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 conditions change occur in the animal (e.g., inability to sustain pregnancy, repeated failure to become pregnant, unhealthy uterus). This will be determined in consultation with the AV.

NO - Please see the non-survival procedure for the mother outlined in the non-survival surgery attachment below.

195.0.14 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 will take place in a surgery suite located in the [redacted] CompMed vivarium between 8am and 4pm, Monday-Friday

195.0.15 Describe the entire surgical procedure.
The animal will be fasted overnight prior to surgery. Once prepped as outlined in Section 194.0.25, 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 a maternal saphenous catheter. Blood chemistry will be evaluated using iStat analyzer. Prior to surgery, a proper plane of anesthesia will be confirmed by a 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 4-24 cm in length depending on gestational age. Once the abdomen is open, the uterus is externalized and examined for the placenta location. The 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. The fetal crown is localized and a horizontal incision is made in the uterus to remove the fetus. A lap sponge may 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 outlined below.

1) Fetus is euthanized and the mother recovers: The fetus is euthanized by injecting the umbilical artery with Beuthanasia-D solution. After the fetus expires (no heartbeat), the cord is cut and the placenta is manually delivered. Cardiac stick is used if a second dose of euthanasia solution is needed after the cord has been cut. (This is rarely needed and the fetus is under the initial effects of the euthanasia solution). Fetal tissues are collected (e.g., liver, kidneys, lung, adipose adrenal, gonads, pancreas, skeletal muscle, heart, pituitary, aorta, carotid artery). The placenta is processed for analysis. The uterus is flushed with saline and sutured. Manual massage is used to stimulate contractions. Once closed, the uterus is placed back in the abdomen and the area is 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 sutures are used; therefore, there is no need to remove the sutures at a later date. Surgical glue may be used once the skin is closed. Analgesia is administered (i.e., Flunixin meglumine (Banamine) with Iron Dextran, IM). Anesthesia is stopped and the animal is monitored until swallowing or response to stimuli is present. The animal is extubated and the catheters are removed. The animal is returned to its home cage and continuously monitored until it is sitting upright. Immediate post-op recovery is monitored by the CompMed staff.

2) Fetus and mother are euthanized: The fetus is euthanized by injecting the umbilical artery with Beuthanasia-D solution. After the fetus expires (no heartbeat), the cord is cut and the placenta is manually delivered. Cardiac stick is used if a second dose of euthanasia solution is needed after the cord has been cut. (This is rarely needed and the fetus is under the initial effects of the euthanasia solution). Fetal tissues are collected (e.g., liver, kidneys, lung, adipose adrenal, gonads, pancreas, skeletal muscle, heart, pituitary, aorta, carotid artery). The placenta is processed for analysis. The mother is then euthanized by an IV injection of Beuthanasia-D solution and isoflurane gas is elevated to the highest level to ensure cessation. Upon confirmation of death, maternal tissue samples will be taken (e.g., 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. The live neonate is cleared of mucous, stimulated to breathe, and placed in a warm blanket. The neonate will be reared by the PI/CompMed staff under the guidance of the AV and the approved SOP for rearing neonates. Once the cord is cut, the placenta is processed for analysis. The uterus is flushed with saline and sutured. Manual massage is used to stimulate contractions. Once closed, the uterus is placed back in the abdomen and the area is 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 sutures are used; therefore, there is no need to remove the sutures at a later date. Surgical glue may be used once the skin is closed. Analgesia is administered (Flunixin meglumine (Banamine) with Iron Dextran, IM). Anesthesia is stopped and the animal is monitored until swallowing or response to stimuli is present. The animal is extubated and the catheters are removed. The animal is returned to its home cage and continuously monitored until it is sitting upright. Immediate post-op recovery is monitored by the 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. The live neonate is cleared of mucous, stimulated to breathe, and placed in a warm blanket. The neonate will be reared by the PI/CompMed staff under the guidance of the AV and the approved SOP for rearing neonates. The placenta is processed for analysis. The mother is then euthanized by IV injection of Beuthanasia-D solution and isoflurane gas is elevated to the highest level to ensure cessation. Upon confirmation of death, maternal tissue samples will be taken (e.g., liver, kidneys, lung, adrenal, gonads, pancreas, skeletal muscle, heart, adipose tissue).

Attachment E cont.: Surgical Procedures (2 of 2)

195.0.10 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. CLICK REPEAT AT THE BOTTOM OF THE PAGE WHEN DONE FILLING INFORMATION ON ONE SURGICAL PROCEDURE TO SAVE AND OPEN A NEW PAGE FOR ANOTHER PROCEDURE AS NEEDED.

195.0.11 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.)
Minor Surgical Procedure

195.0.12 Is the procedure survival surgery?
Yes

195.0.13 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 protocol is designed to evaluate a change in response over time and the function and impact on metabolic and vascular function over adolescent growth through puberty and into adulthood.

Each animal may undergo up to three (3) muscle biopsy procedures over a course of 15 years, (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 (e.g., reduction in movement, adverse change in behavior) that would not be compatible with performing further surgeries.

195.0.14 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 will take place in a surgery suite located in the [redacted] CompMed vivarium between 8am and 4pm, Monday-Friday

195.0.15 Describe the entire surgical procedure.
Initial sedation will be achieved using ketamine (10-15mg/kg), IM.

The baboon is fasted overnight and sedated with ketamine. The animals is intubated and O2 is delivered. The backs 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. The animal is monitored for vitals (e.g., HR, BP, CO2, RR, and temperature) by the 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.

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 baseline blood samples are collected and blood glucose and blood chemistries/gas levels are 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. A sample is taken at ~24-30 months and 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, 30, and 60 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, and respiration, and it is warmed via a warming blanket. Samples are taken to measure a rise in insulin levels and to 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 to relate the findings to insulin sensitivity/resistance as determined by the ivGTT. At the completion of the experiment, the animal is given 1cc (10mg/kg) of iron dextran, IM. The catheters are removed and the animal is monitored for swallowing reflex and response to stimuli before it is extubated. The animal is then returned to its cage.

For muscle biopsy at FMD with infusion of pharmaceutical agent, the procedure is the same pre- and post-infusion of the agent.

Animals will be given Flunixin (2mg/kg, IM) or Ketoprofen (75mg, PO) post-procedure and 2 days post-procedure. Monitoring will be for 5-7 days post-procedure. All records will be kept in the CompMed medical record.

Chair Signature
- Submitted 05/07/2021 10:38 AM ET by [redacted]

chair signature

10.10 By entering your password below, you are electronically signing this protocol.

Signed Friday, May 7, 2021 10:38:16 AM ET by [redacted]

This record is authorized by law (7 USC 2131-2156). Failure to maintain this record can result in a suspension or revocation of license and/or imprisonment for not more than 1 year, or a fine of not more than $ 1,000, or both. [Form approved OMB NO. 0579-0036

U.S. Department of Agriculture Animal and Plant Health Inspection Service

RECORD OF ACQUISITION, DISPOSITION OR TRANSPORT OF ANIMALS (other than Dogs and Cats)

SALE__X__EXCHANGE OR TRANSFER_____DONATION_____

1. Invoice No.

2. Page 1 of _1__

3. Date of Dispostion 10/19/2011

INSTRUCTIONS: Complete applicable items 1 through 13. Original and one copy to accompany animals. When delivery is made - Items 14 through 20 must be completed. Original retained by Buyer (Receiver) and copy returned to Dealer (Seller or Donor). Attach Continuation Sheet (APHIS FORM 7020A) as needed.

4. Dealer's License No. [redacted]

5. Seller or Donor (Name, address, zip, phone #)

Texas Biomedical Research Institute
7620 NW Loop 410
San Antonio, Texas 78227
[redacted]

6. Buyer or Receiver (Name, address, zip, phone #)

EVMS, Dept of Comparative Med
[redacted] Norfolk VA 23507

7. USDA license No (if any)

8. IDENTIFICATION OF ANIMALS BEING DELIVERED

Container Tag No., Crate or Pen No.	Number Animals	Previous Invoice No. (if any)	Individual Ident., Tatoos, Tag Nos. (if applicable)	Species	Age-Sex: Number Young M	Age-Sex: Number Young F	Age-Sex: Number Adult M	Age-Sex: Number Adult F	Est. Weight (kgs)	Remarks (Condition, etc.)	Receiver Use	Receiver Use
1	1		26741	PCA				1	14.12			
[redacted]												
3	1		26876	PCA				1	14.4			
[redacted]												

DELIVERY BY COMMERCIAL CARRIER

9. Delivery by: ____Buyer's Truck _____Dealer's Truck (Seller or Donor)	10. Truck License No.	11. Bill of Lading No.

12. Name, address, phone of Company or Firm	13. Name, address, phone of Truck Driver
[redacted]	

DELIVERY RECEIPT - TO BE COMPLETED BY BUYER OR RECEIVER

14. Animals Delivered were ("x" one)	____in apparent good condition	____ poor condition _____rejected
15. Total number received		attach explanation for rejection
16. Number Dead	17. Number Alive	
18. BY (signature)	19. Title	20. Date

APHIS FORM 7020 (APR 93)

(replaces VS FORM 18-20, which is now obsolete, and APHIS Form 7020 (10-90) which may be used.)

TEXAS B[redacted]MEDICAL RESEARCH INSTIT[redacted]E

7620 N.W. Loop 410
San Antonio, Texas 78227

Veterinary Health Certificate
Date: October 19, 2011

Consignor	**Consignee**
Texas Biomedical Research Institute 7620 NW Loop 410 San Antonio, Texas 78227	Eastern Virginia Medical School Dept of Comparative Medicine [redacted] [redacted] Norfolk, VA 23507
[redacted] Attending Veterinarian	[redacted]

Description of Animal(s)

Sex	Weight	Tattoo/Tag Number	Species	Last TB Test	Date of Birth
F	14.12	26741	PCA	10/11/2011	6/1/2005
[redacted]					
F	14.4	26876	PCA	10/11/2011	6/29/2005
[redacted]					

Certification: I hereby certify that I have this date examined the animal(s) listed above and found them, to the best of my knowledge, to be free of clinical symptoms of any infectious or communicable disease.

Statement of Acclimatization: These animals are acclimatized to a temperature of 40° F. They should not, however, be exposed for over 30 minutes.

Remarks: [redacted]

TB/PHYSICALS/WEIGHTS

ANIMAL ID/NAME: 26741 / Tara

INVESTIGATOR: [redacted]

ENTRY PE: 10-21-11 **EXIT PE:**

Date	Weight (kg)	TB	PE	OTH	Comments	Initial
[redacted]						
22Jan21	18.1	✓	✓		TB/PE (Preg)	JH
20210121	15.4	X	X			SM

Chronological Summary Sheet

Principal Investigator	Room No.	Protocol No.	Animal Number
[redacted]	[redacted]	21-003	26741
Vendor	**DOA/DOB**	**Date of Euthanasia**	**Species/Sex**
SWFBR	10-20-11		Boon

Entry Date	Treatment and Observation	Initials
5.6.21	Transfer to 21-003	[redacted]
20JUL21	Animal sedated with 1.6 ml ketamine IM for PE, blood collection, and TB test. Normal recovery.	
	TB ⊖ [redacted]	[redacted]

All USDA regulated species Enrichment Records are in a different location

Chronological Summary Sheet

Principal Investigator	Room No.	Protocol No.	Animal Number
[redacted]	[redacted]	18-006	26741 / Tara
Vendor	**DOA/DOB**	**Date of Euthanasia**	**Species/Sex**
SWFBR	10/20/2011		Baboon / F

Entry Date	Treatment and Observation	Initials
[redacted]		
22 Jan 24	Animal sedated with 1.5 ml Ketamine IM for PE & TB test. Normal recovery. TB results came back negative.	
	[redacted]	

All USDA regulated species Enrichment Records are in a different location

MASTER PROBLEM/EVENTS

Animal ID/Name:	26741 / Tara	Investigator:	[redacted]

Date	Clinical Call or Event	Initials	Date of Resolution	Initials
[redacted]				
22Jan21	TB / PE	[redacted]	25 Jan 21	[redacted]
20JU121	TB/PE		23JU121	

ANTECH

800-872-1001

Patient Info:
Name: TARA
Chart No: 26741
Owner: [redacted]
Doctor: [redacted]

Species: Primate
Breed: Baboon
Age: 10Years
Sex: F

Hospital:
Eastern VA Med ATT [redacted]
[redacted]
Norfolk, VA 23507

Lab:
ANTECH Diagnostics
3675 Concorde Pkwy
Chantilly, VA 20151
Reported: 07/22/21 04:40 AM
Received: 07/21/21

Antech ID: [redacted]

Accession No.	Doctor	Owner	Pet Name
ROA			TARA

Test	Results	Reference Range	L / Normal / H
Superchem			
Total Protein	6.4	5.0-7.0 g/dL	
Albumin	**3.8**	**2.4-3.3 g/dL**	**HIGH**
Globulin	2.6	2.0-4.0 g/dL	
A/G Ratio	**1.5**	**0.5-1.0**	**HIGH**
AST (SGOT)	22	10-100 IU/L	
ALT (SGPT)	22	10-100 IU/L	
Alk Phosphatase	141	100-250 IU/L	
GGTP	23	1-25 IU/L	
Result Verified			
Total Bilirubin	0.1	0.0-1.0 mg/dL	
Urea Nitrogen	15	10-22 mg/dL	
Creatinine	0.7	0.1-2.0 mg/dL	
BUN/Creatinine Ratio	21		
Phosphorus	4.5	3.0-5.5 mg/dL	
Glucose	70	70-120 mg/dL	
Calcium	9.3	7.2-11.5 mg/dL	
Magnesium	1.4	mEq/L	
Sodium	146	138-150 mEq/L	
Potassium	3.9	3.5-5.5 mEq/L	
NA/K RATIO	37		
Chloride	**108**	**109-115 mEq/L**	**LOW**
Cholesterol	**87**	**100-200 mg/dL**	**LOW**
Triglycerides	56	28-109 mg/dL	
Amylase	**97**	**1000-2500 IU/L**	**LOW**
PrecisionPSL™	13	U/L	
CPK	**440**	**100-400 IU/L**	**HIGH**
Complete Blood Count			
WBC	**16.1**	**8.0-16.0 $10^3/\mu L$**	**HIGH**
RBC	**4.9**	**5.50-8.50 $10^6/\mu L$**	**LOW**
Hemoglobin	**12.5**	**14.5-17.0 g/dL**	**LOW**
HCT	42	38.0-50.0 %	
MCV	**84**	**65-80 fL**	**HIGH**
MCH	25.4	23.0-26.0 pg	
MCHC	**30**	**32.0-35.0 g/dL**	**LOW**
Blood Parasites	None Seen		
RBC Comment			
RBC Morphology	Normal		
Platelet Count	365	$10^3/\mu L$	
Platelet EST	Adequate		

Differential	**Absolute**	**%**		
Neutrophils	**13202**	**82**	**4800-12000 /µL**	**HIGH**
Bands		0		
Lymphocytes	2415	15	1600-4800 /µL	
Monocytes	**483**	**3**	**0-480 /µL**	**HIGH**
Eosinophils	**0**	**0**	**80-800 /µL**	**LOW**
Basophils	0	0	/µL	

Comment
Blood smear reviewed by technologist.

REVIEWED
By [redacted] at 9:14 am, Jul 22, 2021

FINAL

TB Test Worksheet

Date of Test: 20 Jul 21 Test Site (circle one): L eye **R eye** Abdomen

Species: Baboons Room Tested: [redacted]

Test Reaction Grades		
Reaction	Grade	Description of Changes
0	Neg	No Reaction
1	Neg	Bruise associated with the injection
2	Neg	Erythema-no swelling, serious bruising or reaction
3	Suspect	Minimal swelling w/ or w/o erythema
4	Pos	Obvious swelling, drooping eyelid. Some degree of erythema
5	Pos	Swelling &/or necrosis eyelid closed

			Date: 21 Jul 21		Date: 22 Jul 21		Date: 23 Jul 21	
Animal ID	Animal Name	Wt (kg)	24hr	Initials	48hr	Initials	72hr	Initials
[redacted]								
26741	Tara		0	[redacted]	0	[redacted]	0	[redacted]
[redacted]								
				[redacted]		[redacted]		

[redacted]

Physical Exam

Date: 20 Jul 2021

Animal Name: Tara

Animal ID: 26741

Species: Baboon

Sex: M / (F)

Investigator: [redacted]

Initial Ketamine: 1.6 cc

+ Add. Used: _____ cc

= Total Ketamine: _____ cc

Animal Weight: 15.4 kg

Physical Type: ☐ Entry ☐ Exit ☐ Annual ☑ Biannual

Physical Findings

Overall — Temp: 100 deg F | Pulse: 122 bpm | Resp: 44 br./min

EENT:	☒ Normal	☐ Abnormal
Head:	☒ Normal	☐ Abnormal
Heart/Lungs:	☒ Normal	☐ Abnormal
Abdomen:	☒ Normal	☐ Abnormal
Lymph Nodes:	☒ Normal	☐ Abnormal
Arms/Hands:	☐ Normal	☒ Abnormal *
Legs/Feet:	☐ Normal	☒ Abnormal *
Genitals:	☒ Normal	☐ Abnormal
Coat/Tail:	☒ Normal	☐ Abnormal #

Dentition
(Grade 0-5): Tartar: 2 Gingivitis: 2

Notes/Missing or Abnormal Teeth: - Silver teeth / Bar Biting. Small Gum abrasion -

Dental Needed? ☒ Yes

Notes/Comments:

TB TEST ADMINISTERED ☒

* Missing digits R Foot / R. Hand. # Some alopecia.

BCS: 3

Dental: Cleaning needed. Prevent Tartar buildup. R/L Saphenous Vein blood draws.

Laboratory Workup: ☐ None X CBC/Chemistry ☐ Fecal

Lab. Used: Antech ☐ SRS/SIV ☐ Herpes B ☐ FILO

[redacted] 7/21/21

Performed by: [redacted]

Physical Exam

Date: 22 Jan 2021

Animal Name: Tara

Animal ID: 26741

Species: Baboon

Sex: M / (F)

Investigator: [redacted]

Initial Ketamine: 1.5 cc

+ Add. Used: _____ cc

= Total Ketamine: 1.5 cc

(1.3 (incl 0.2

Animal Weight: 18.1 kg

Physical Type: ☐ Entry ☐ Exit ☐ Annual X Biannual

Physical Findings

Overall — Temp: 101.3 deg F | Pulse: 100 bpm | Resp: 40 br./min

E E N T:	☒ Normal	☐ Abnormal
Head:	☒ Normal	☐ Abnormal
Heart/Lungs:	☒ Normal	☐ Abnormal
Abdomen:	☐ Normal	☒ Abnormal
Lymph Nodes:	☒ Normal	☐ Abnormal
Arms/Hands:	☐ Normal	☒ Abnormal
Legs/Feet:	☒ Normal	☐ Abnormal
Genitals:	☒ Normal	☐ Abnormal
Coat/Tail:	☐ Normal	☒ Abnormal

Dentition (Grade 0-5): Tartar: 2 Gingivitis: 1

Notes/Missing or Abnormal Teeth:

[redacted]

Dental Needed? ☒ Yes

Notes/Comments:

TB TEST ADMINISTERED ☒ (L)

Pregnant

Missing 5th digit on (R) hand

Alopecia on abdomen

Laboratory Workup: ☒ None ☐ CBC/Chemistry ☐ Fecal

Lab. Used: ✓ ☐ SRS/SIV ☐ Herpes B ☐ FILO

Performed by: [redacted]

TB Test Worksheet

Date of Test: 22 Jan 21 Test Site (circle one): (L eye) R eye Abdomen

Species: Baboons Room Tested: [redacted]

Test Reaction Grades	
Reaction Grade	Description of Changes
0----------Neg	No Reaction
1---------- Neg	Bruise associated with the injection
2---------- Neg	Erythema-no swelling, serious bruising or reaction
3---------- Suspect	Minimal swelling, w/ or w/o erythema
4---------- Pos	Obvious swelling, drooping eyelid. Some degree of erythema
5---------- Pos	Swelling &/or necrosis eyelid closed

			Date: 23 Jan 21		Date: 24 Jan 21		Date: 25 Jan 21	
Animal ID	Animal Name	Wt (kg)	24hr	Initials	48hr	Initials	72hr	Initials
26741	Tara	15.5	0	[redacted]	0	[redacted]	0	[redacted]
26876	Jemma	14.0	0		0		0	
[redacted]								
28768	Juju	15.1	0		0		0	
[redacted]								

This record is authorized by law (7 USC 2131-2166). Failure to maintain this record can result in a suspension or revocation of license and/or imprisonment for not more than 1 year, or a fine of not more than $ 1,000, or both. [Form approved OMB NO. 0579-0036

U.S. Department of Agriculture Animal and Plant Health Inspection Service

RECORD OF ACQUISITION, DISPOSITION OR TRANSPORT OF ANIMALS (other than Dogs and Cats)

SALE___EXCHANGE_X__ OR TRANSFER_____DONATION_____

1. Invoice No.

2. Page 1 of _1__

3. Date of Dispostion 2/20/2012

INSTRUCTIONS: Complete applicable items 1 through 13. Original and one copy to accompany animals. When delivery is made - Items 14 through 20 must be completed. Original retained by Buyer (Receiver) and copy returned to Dealer (Seller or Donor). Attach Continuation Sheet (APHIS FORM 7020A) as needed.

4. Dealer's License No. [redacted]

5. Seller or Donor (Name, address, zip, phone #)

Texas Biomedical Research Institute
7620 NW Loop 410
San Antonio, Texas 78227
[redacted]

6. Buyer or Receiver (Name, address, zip, phone #)

EVMS, Dept of Comparative Med
[redacted] Norfolk VA 23507

7. USDA license No (if any)

8. IDENTIFICATION OF ANIMALS BEING DELIVERED

Container Tag No., Crate or Pen No.	Number Animals	Previous Invoice No. (if any)	Individual Ident., Tatoos, Tag Nos. (if applicable)	Species	Age-Sex: Number Young M	Age-Sex: Number Young F	Age-Sex: Number Adult M	Age-Sex: Number Adult F	Est. Weight (kgs)	Remarks (Condition, etc.)	Receiver Use	Receiver Use
1	1		28768	PCA				1	16.05			
[redacted]												

DELIVERY BY COMMERCIAL CARRIER

9. Delivery by: ____Buyer's Truck _____Dealer's Truck (Seller or Donor)	10. Truck License No.	11. Bill of Lading No.

12. Name, address, phone of Company or Firm	13. Name, address, phone of Truck Driver
[redacted]	

DELIVERY RECEIPT - TO BE COMPLETED BY BUYER OR RECEIVER

14. Animals Delivered were ("x" one)	____in apparent good condition	____ poor condition _____rejected
15. Total number received		attach explanation for rejection
16. Number Dead	17. Number Alive	
18. BY (signature)	19. Title	20. Date

APHIS FORM 7020
(APR 93)

(replaces VS FORM 19-20, which is now obsolete, and APHIS Form 7020 (10-90) which may be used.)

TEXAS B[redacted] MEDICAL RESEARCH INSTIT[redacted]TE

7620 N.W. Loop 410
San Antonio, Texas 78227

Veterinary Health Certificate
Date: February 20, 2012

Consignor	**Consignee**
Texas Biomedical Research Institute 7620 NW Loop 410 San Antonio, Texas 78227	Eastern Virginia Medical School Dept of Comparative Medicine [redacted] Norfolk, VA 23507
[redacted] Attending Veterinarian	[redacted]

Description of Animal(s)

Sex	Weight	Tattoo/Tag Number	Species	Last TB Test	Date of Birth	
F	16.05	28768	PCA	1/27/2012	3/26/2007	
[redacted]						

Certification: I hereby certify that I have this date examined the animal(s) listed above and found them, to the best of my knowledge, to be free of clinical symptoms of any infectious or communicable disease.

Statement of Acclimatization: These animals are acclimatized to a temperature of 40° F. They should not, however, be exposed for over 30 minutes.)

Remarks: [redacted]

TB/PHYSICALS/WEIGHTS

ANIMAL ID/NAME: 28768/ JNJ1

INVESTIGATOR: [redacted]

ENTRY PE: 2.22.12 EXIT PE: _____

Date	Weight (kg)	TB	PE	OTH	Comments	Initial
1/25/17	[redacted]					
22Jan21	17.4	✓	✓	X	TB/PE (Preg)	
09MAR21	–	–	–	✓	SEA CHECK/ALOPECIA	
20JUL21	16.7	X	X	X	TB, PE, dental	

Chronological Summary Sheet

Principal Investigator	Room No.	Protocol No.	Animal Number
[redacted]	[redacted]	21-003	28768 / J-Ju
Vendor	**DOA/DOB**	**Date of Euthanasia**	**Species/Sex**
SWFBR	2-21-12		Baboon / ♀

Entry Date	Treatment and Observation	Initials
20JU121		
	Animal sedated with 2.1 ml ketamine IM for PE, blood collection, and TB test. Normal recovery. + dental	
	TB ⊖ [redacted]	
1-12-22	Animal Found with skin laceration to (L) 2nd index Finger. NO brake presented. Cleaned and glue was applied per AV. Animal under protocol study will be checked daily. See daily sheet.	[redacted]
1-13-22	Finger is healing.	
1-14-22	applied Chlorhexadine to Clean.	
1-15-22	closed.	
1-17-22	Finger has closed - STAGE 0	

All USDA regulated species Enrichment Records are in a different location

Chronological Summary Sheet

Principal Investigator	Room No.	Protocol No.	Animal Number
[redacted]	[redacted]	18-006	28768 / Juju
Vendor	**DOA/DOB**	**Date of Euthanasia**	**Species/Sex**
SWFBR	2-21-12		Baboon / Female

Entry Date	Treatment and Observation	Initials
22Jan21	Animal sedated with 1.5 ml Ketamine IM for PE & TB test. Normal recovery. TB results came back negative.	[redacted]
09MAR21	SEA CHECK	
	ABSCESS ON (L) ARM & FOREARM MEDIAL SIDE	
4/14/21	Sedated with ketamine for fetal injection. Maintained w/ iso. Normal recovery.	
15Apr21	P sedated with 1.5ml ketamine IM for C-section under isoflurane per protocol. Normal recovery see anesthesia sheet for more details.	
5-6-21	Transfer to 21-003	

[redacted]

All USDA regulated species Enrichment Records are in a different location

MASTER PROBLEM/EVENTS

Animal ID/Name:	28768/ JUJU	Investigator:	[redacted]

Date	Clinical Call or Event	Initials	Date of Resolution	Initials
[redacted]				
7/25/17				
22Jan21	TB / PE (Preg)		25Jan21	
09MAR21	ALOPECIA ON (L) ARM & FOREARM			
4/14/21	Fetal injection			
15Apr21	C-section			
20JUL21	TB, PE, dental		23JUL21	

POST OPERATIVE EVALUATION FORM

Species: Baboon

Date of Operation: 4-15-21 **Protocol #:** 18-006

Pre-Operative Weight: 17.4Kg **Animal Id** 28768 JWU

Pain/Distress Assessment Scoring:
0= *No signs of pain or distress* (BAR with normal defecation and urination)
1= *Minimal signs of pain or distress* (BAR to QAR, subtle behavioral/physiological changes i.e. decreased defecation, reaction upon palpation of site, guarding site)
2= *Moderate signs of pain or distress* (BAR, QAR to recumbent or other behavioral changes associated with pain or distress i.e. slow movement, guarding of incision, vocalization/aggression. There may swelling/redness/discharge at site)
3= *Marked sings of pain or distress* (Recumbent, vocal, decreased defecation, urination and appetite. +/-dehiscence of incision)
4= Agonal, moribound or comatose/unresponsive
****Anything above a "0" requires reassessment of analgesic regimen and contacting the attending veterinarian****

Post -op Date	4-15-21		4-16-21		4-17-21		4-18-21		4-19-21		4-20-21		4-21-21	
Time of Day		330pm	9:00AM	3pm	7:35AM	5pm	6:30A		8:55AM		9:00AM	12:30	8:30A	
Active (Bar/Qar)		BAR	BAR	BAR	BAR	BAR	BAR		BAR		BAR	BAR	BAR	
Inquisitive		Y	Y	Y	Y	Y	Y		Y		Y	Y	Y	
Urination		Y	Y	Y	Y	Y	Y		Y		Y	Y	Y	
Feces		Y	Y	Y	Y	Y	Y		Y		Y	Y	Y	
Evidence of Eating		Y	Y	Y	Y	Y	Y		Y		Y	Y	Y	
Evidence of Drinking		Y	Y	Y	Y	Y	Y		Y		Y	Y	Y	
Gait/Posture NR		NR	NR	NR	NR	NR	NR		NR		NR	NR	NR	
Incision Edge(s) Red		1	2	2	2	2	2		2		2	2	2	
Sutures Intact		Y	Y	Y	Y	Y	Y		Y		Y	Y	Y	
Swelling present		2	2	2	2	2	2		2		2	2	2	
Exudate from incision		2	2	2	2	2	2		2		2	2	2	
Pain Score (0-4)**		1	0	0	0	0	0		0		0	0	0	
Medication: Ketamine **Concentration Mg/ml:** **Dosage cc:** 0.2mg/kg	See Anesthesia sheet	0.7mL	0.7ml	0.7mL	0.7ml	0.7mL	0.7mL		/		/	/	/	
Medication: **Concentration Mg/ml** **Dosage in cc:**														
Comments		notified Vet.					Vaginal Bleeding-NORMAL							4/16/21
Initials		[redacted]												
CompMed review														

Created 9-2015; Revised 6-2016

EVMS CompMed
ANIMAL MEDICAL RECORD OPERATIVE REPORT

General Information

Animal Id: 28768 JuJu TCGS E2 FID162

Date/Time Field: April 14&15, 2021

Investigator: [redacted]

Protocol #: 18-006

Sex: f

Species/breed/strain: Baboon

Name(s) of Surgeon(s) and Assistant(s) [Personnel listed must have completed appropriate training and must be listed on the approved IACUC protocol]

[redacted]

Name (s) of Anesthesiologist

[redacted]

Surgery/Procedure description

[Description must be sufficiently detailed to facilitate examination of the animal by the veterinarian or designee]

Term CGS pregnancy with fetal injection of 10ug estradiol + estradiol benzoate On April 14th, animal was sedated with ketamine (IM), The fetus was located using ultrasound. 10ug of estradiol and estradiol benzoate was injected into the shoulder. The mother recovered and was returned to her cage. 24hrs later, animal was sedated and intubated. Isoflurane was used during the surgery. A vertical incision was made to the abdomen and the uterus was externalized. Blood samples were taken from uterine veins. The uterus was opened and the fetus was removed. Blood samples were taken from the umbilical cord. The cord clamped and fatal plus delivered via umb V. The cord was cut once fetus was euthanized. Tissues were taken from the fetus. The placenta was delivered and appeared normal. The uterus flushed with sterile saline. Using 2-0 PDS suture, the uterus was closed with simple interrupted knots. The peritoneum/fascia was closed using 2-0 PDS and a cruciate suture pattern. The fascia was then closed with a running suture line. Finally the skin was closed using 3-0 PDS subcuticular.

Description of intra-operative complications, if applicable (or comments)

No complications regarding the procedure. Notes: No major adhesions or abnormalities within the abdomen. No major or concerning issues. Animal recovered from anesthesia as expected is being monitored post-operatively as outlined in the protocol. Post-surgical analgesia was given on the table after the fetus was removed and followed as outlined in the approved protocol.

Medications administered relevant to intra-operative complications &/or emergencies
(Please list Drug name, Dose and Route plus any applicable comments)

None

Signature Field: [redacted]

Treatment protocol for baboon # 28768 Juju — year 2021

body weight = 18.0kg

	date	CGS (ml)	Ketamine (cc)	Vit	Initials	blood sample from m.s. vein 3-5ml
105	2/17/21	0.5mL	1.5		[redacted]	✓
106	2/18/21	1.0mL	1.5			✓
107	2/19/21	1.0ml	1.5			✓
108	2/20/21	1.2ml	1.6			✓
109	2/21/21	1.2	1.6			✓
110	2/22/21	1.5	1.2			✓
111	2/23/21	1.0	1.5			✓
112	2/24/21	1.0	1.7			✓
113	2/25/21	1.5	2.0			✓
114	2/26/21	1.0	2.0			✓
115	2/27/21	1.0	1.8			✓
116	2/28/21	1.0	1.8			✓
117	3/1/21	1.3	2.0			✓
118	3/2/21	1.4	2.0			✓
119	3/3/21	1.4	2.0			✓
120	3/4/21	1.4	2.0			✓
121	3/5/21	1.5	2.0			✓
122	3/6/21	1.5				
123	3/7/21	1.5	2.0			✓
124	3/8/21	1.5	2.0			✓
125	3/9/21	1.5				
126	3/10/21	1.5	2.0			✓
127	3/11/21	1.5	2.0			
128	3/12/21	1.5	1.8			✓
129	3/13/21	1.5	1.8			✓
130	3/14/21	1.5				
131	3/15/21	1.5	1.8			
132	3/16/21	1.5	1.9			✓
133	3/17/21	1.5	2.0			✓
134	3/18/21	1.5	2.0			✓
135	3/19/21	1.5	1.8			
136	3/20/21	1.5	1.7			
137	3/21/21	1.0	2.0			
138	3/22/21	1.0	1.9			✓
139	3/23/21	1.0	2.0			
140	3/24/21	1.0	1.9			
141	3/25/21	1.0	2.0			✓ ✓
142	3/26/21	1.0	~~8~~			
143	3/27/21	1.0				
144	3/28/21	1.0	CM			✓
145	3/29/21	1.0				
146	3/30/21	1.0				
147	3/31/21	1.0				
148	4/1/21	1.0	2.0			✓
149	4/2/21	1.0	2.0			✓

	Treatment protocol for baboon # 28768 Juju				year 2021		
					blood sample from m.s. vein		
		CGS (ml)	Ketamine (cc)	Vit	Initials	3-5ml	
150	4/3/21	1.0ml	2.0		[redacted]	✓	
151	4/4/21	1.0ml	2.0				
152	4/5/21	1.0	2.0			✓	
153	4/6/21	0.75	2.0			✓	
154	4/7/21	0.75	1.8			✓	
155	4/8/21	0.75	1.8			✓	
156	4/9/21	0.75	1.9			✓	
157	4/10/21	0.75					
158	4/11/21	0.7C					
159	4/12/21	1.0	1.3			✓	
160	4/13/21	1.0	2.0			✓	
161	4/14/21	1.0ml	2.0			✓	FI
162	4/15/21		2.0				SX
163	4/16/21						
164	4/17/21						
165	4/18/21						
166	4/19/21						
167	4/20/21						
168	4/21/21						
169	4/22/21						
170	4/23/21						
171	4/24/21						
172	4/25/21						
173	4/26/21						
174	4/27/21						

EVMS Comparative Medicine Division
NHP Surgery/Anesthesia Form

Date: 4/15/21 **Animal ID:** 28768 - Juju **Weight (Kg):** 17.4 **Protocol:** 18-006
Procedure/Surgery: C-section **Current wgt:** **Investigator:** [redacted]
Surgeon: [redacted]
Anesthetist:

Presurgical Treatment	Dose	Amt.	Route	Time	Init.

Induction	mg/kg	calc (ml)	Amt.	Route	Time	Init.
Ketamine	10	1.74	1.5	IM	8:30	[redacted]
Glycopyrolate	0.006	0.522				
Ketamine/Xylazine	[7 / 0.6]					

Endotracheal Tube			T Intub.	T Extub
			8:50	10:40
Anesthesia		**Type**	**T Start**	**T End**
		Isoflurane	8:50	10:23
Fluids	**Type**	**ml/hour ***	**T Start**	**T End**
	NaCl	6.96	8:57	10:25
Procedure/Surgery			**T Start**	**T End**
			9:25	10:25

* maintenance rate of 2 ml/kg/hr

Comments:
Banamine 0.7ml IM @ 9:55 [redacted]
From 1.5ml IM @ 10:20 [redacted]

5.5 [redacted]

Time	HR	SpO_2	CO_2%	RR	Iso%	BP Sys/Dia	MAP	Temp	Treatments or comments	Time	Init
9:00	99	97	4.5	19	2.5	107/59	76	98.6	ISO ↓ 2%	9:15	[redacted]
9:08	87	99	4.7	12	2.5	~~107/59~~		97.9	54/27 (35)		
9:18	83	100	5.0	12	2.0	99/32	41	97.5			
9:25	86	99	5.2	13	2.0	52/28	40	97.3	SX start		
9:30	92	100	4.9	14	2.0	52/19	36	97.2			
9:35	91	100	4.7	15	2.0	53/27	38	97.1			
9:40	95	100	4.8	14	2.0	58/28	40	97.0			
9:45	100	100	5.0	15	2.0	62/30	42	97.0			
9:55	106	99	4.7	11	2.0	70/25	41	96.4	Begin suture	9:50	
10:05	99	99	4.5	11	2.0	62/25	40	96.0			
10:15	89	100	4.7	10	2.0	58/23	39	95.9	ISO ↓ 1.5%, begin skin		
10:20	90	99	4.4	11	1.5	57/33	43	95.7	ISO ↓ 1%		
10:23	98	99	4.4	12	1.0	65/26	42	95.7	ISO off		
10:40	–	–	–	–	–	–	–	–	Chewing, jaw tone		
10:45	–	–	–	–	–	–	–	–	blinking, RTC		
11:15	–	–	–	–	–	–	–	–	upright, RTS, alert		

Postsurgical Treatment	Dose	Amt.	Route	Time	Init.

[redacted] 19APR21

Emergency Drugs	mg/kg	calc'd	Amt.	route	time	initials	Comments
Atropine	0.02	0.87					max 2mg/kg ↑ Heart Rate
Doxapram	2	1.74					Stimulates Respiration
Epinephrine 1:100	0.2	3.48					0.1-0.5mg/kg Improves breathing, Stimulates Heart, ↑ BP
Glycopyrrolate	0.004	0.348					
Lidocaine	1	0.174					max 3mg/kg
Prednisolone Sodi	10	8.7					
Phenylephrine	n/a	0.2 mg		IV			do not give more than q10 min - think of a continuous drip.

ANTECH

800-872-1001

Patient Info:
Name: JUJU
Chart No: 28768
Owner: [redacted]
Doctor: [redacted]

Species: Primate
Breed: Baboon
Age: 14Years
Sex: F

Hospital:
Eastern VA Med ATT [redacted]
[redacted]
Norfolk, VA 23507

Antech ID: [redacted]

Lab:
ANTECH Diagnostics
3675 Concorde Pkwy
Chantilly, VA 20151
Reported: 07/22/21 01:25 AM
Received: 07/21/21

Accession No.	Doctor	Owner	Pet Name
RO[redacted]			JUJU

Test	Results		Reference Range	L / Normal / H
Superchem				
Total Protein	6.5		5.0-7.0 g/dL	
Albumin	**4.0**		**2.4-3.3 g/dL**	**HIGH**
Globulin	2.5		2.0-4.0 g/dL	
A/G Ratio	**1.6**		**0.5-1.0**	**HIGH**
AST (SGOT)	30		10-100 IU/L	
ALT (SGPT)	23		10-100 IU/L	
Alk Phosphatase	**81**		**100-250 IU/L**	**LOW**
GGTP	**34**		**1-25 IU/L**	**HIGH**
Result Verified				
Total Bilirubin	0.1		0.0-1.0 mg/dL	
Urea Nitrogen	14		10-22 mg/dL	
Creatinine	0.8		0.1-2.0 mg/dL	
BUN/Creatinine Ratio	18			
Phosphorus	3.5		3.0-5.5 mg/dL	
Glucose	**55**		**70-120 mg/dL**	**LOW**
Calcium	8.9		7.2-11.5 mg/dL	
Magnesium	1.5		mEq/L	
Sodium	142		138-150 mEq/L	
Potassium	**3.1**		**3.5-5.5 mEq/L**	**LOW**
NA/K RATIO	46			
Chloride	**103**		**109-115 mEq/L**	**LOW**
Cholesterol	**99**		**100-200 mg/dL**	**LOW**
Triglycerides	41		28-109 mg/dL	
Amylase	**156**		**1000-2500 IU/L**	**LOW**
PrecisionPSL™	22		U/L	
CPK	382		100-400 IU/L	
Complete Blood Count				
WBC	**6.4**		**8.0-16.0 10^3/µL**	**LOW**
RBC	**3.9**		**5.50-8.50 10^6/µL**	**LOW**
Hemoglobin	**9.7**		**14.5-17.0 g/dL**	**LOW**
HCT	**32**		**38.0-50.0 %**	**LOW**
MCV	**82**		**65-80 fL**	**HIGH**
MCH	25.2		23.0-26.0 pg	
MCHC	**31**		**32.0-35.0 g/dL**	**LOW**
Blood Parasites	None Seen			
RBC Comment				
RBC Morphology	Normal			
Platelet Count	321		10^3/µL	
Platelet EST	Adequate			
Differential	**Absolute**	**%**		
Neutrophils	5440	85	4800-12000 /µL	
Bands		0		
Lymphocytes	**768**	12	**1600-4800 /µL**	**LOW**
Monocytes	192	3	0-480 /µL	
Eosinophils	**0**	**0**	**80-800 /µL**	**LOW**
Basophils	0	0	/µL	

Comment
Blood smear reviewed by technologist.

APPROVED
By [redacted] at 10:08 am, Jul 22, 2021

FINAL

For online lab results please visit online.antechdiagnostics.com

TB Test Worksheet

Date of Test: 20 Jul 21 Test Site (circle one): L eye **R eye** Abdomen

Species: Baboons Room Tested: [redacted]

Test Reaction Grades	
Reaction Grade	Description of Changes
0----------Neg	No Reaction
1---------- Neg	Bruise-associated with the injection
2---------- Neg	Erythema-no swelling, serious bruising or reaction
3---------- Suspect	Minimal swelling, w/ or w/o erythema
4---------- Pos	Obvious swelling, drooping eyelid. Some degree of erythema
5---------- Pos	Swelling &/or necrosis eyelid closed

			Date: 21 Jul 21		Date: 22 Jul 21		Date: 23 Jul 21	
Animal ID	Animal Name	Wt (kg)	24hr	Initials	48hr	Initials	72hr	Initials
[redacted]			[redacted]					
26876	Jemma		0	JH	0	JH	0	
28768	Juju		0	JH	0	JH	0	

[redacted]

22 Aug 21

Physical Exam

Date: 20 Jul 2021

Animal Name: Juju

Animal ID: 28768

Species: Baboon

Sex: M / (F)

Investigator: [redacted]

Initial Ketamine: 1.6 cc

+ Add. Used: 0.5 cc + 0.1 xylazine

= Total Ketamine: 2.1 cc

Animal Weight: 16.7 kg

Physical Type: ☐ Entry ☐ Exit ☐ Annual ☑ Biannual

Physical Findings

Overall Temp: 98.7 deg F | Pulse: ~~98~~ 89 bpm | Resp: 36 br./min

EENT:	☒ Normal	☐ Abnormal
Head:	☒ Normal	☐ Abnormal
Heart/Lungs:	☒ Normal	☐ Abnormal
Abdomen:	☒ Normal	☐ Abnormal
Lymph Nodes:	☒ Normal	☐ Abnormal
Arms/Hands:	☐ Normal	☒ Abnormal
Legs/Feet:	☐ Normal	☒ Abnormal
Genitals:	☒ Normal	☐ Abnormal
Coat/Tail:	☐ Normal	☒ Abnormal

Dentition
(Grade 0-5): Tartar: _____ Gingivitis: _____

Notes/Missing or Abnormal Teeth:
Dental performed by [redacted]
[redacted]

Dental Needed? ☐ Yes

Notes/Comments:

TB TEST ADMINISTERED ☒ L.

Missing one left foot digit; missing one left hand digit;

Alopecia (L) shoulder and (L)+(R) arms

Laboratory Workup: ☐ None ☒ CBC/Chemistry ☐ Fecal

Lab. Used: _Antech [redacted]

☐ SRS/SIV ☐ Herpes B ☐ FILO

[redacted] 7/22/21

Performed by: [redacted]

Physical Exam

Date: 22 Jan 2021

Animal Name: Juju

Animal ID: 28768

Species: Baboon

Sex: M / (F)

Investigator: [redacted]

Initial Ketamine: 1.5 cc

+ Add. Used: _____ cc

= Total Ketamine: 1.5 cc (IM)

Animal Weight: 17.4 kg

Physical Type: ☐ Entry ☐ Exit ☐ Annual X Biannual

Physical Findings

Overall

Temp: 100.5 deg F | Pulse: 128 bpm | Resp: 48 br./min

E E N T:	☒ Normal	☐ Abnormal
Head:	☒ Normal	☐ Abnormal
Heart/Lungs:	☒ Normal	☐ Abnormal
Abdomen:	☐ Normal	☒ Abnormal
Lymph Nodes:	☒ Normal	☐ Abnormal
Arms/Hands:	☐ Normal	☒ Abnormal
Legs/Feet:	☐ Normal	☒ Abnormal
Genitals:	☒ Normal	☐ Abnormal
Coat/Tail:	~~☒ Normal~~	☒ Abnormal

Dentition
(Grade 0-5): Tartar: 1 Gingivitis: 1

Notes/Missing or Abnormal Teeth:

Dental Needed? ☐ Yes (NO)

[redacted] 1/25/21

Notes/Comments:

TB TEST ADMINISTERED ☒ (6)

(R) hand fourth finger distal tip missing; alopecia (L) + (R) arms

(R) ~~feed~~ foot 4th toe distal tip missing

Pregnant

Laboratory Workup: ☒ None ☐ CBC/Chemistry ☐ Fecal

Lab. Used: _____ ☐ SRS/SIV ☐ Herpes B ☐ FILO

Performed by: [redacted]

TB Test Worksheet

Date of Test: 22 Jan 21 Test Site (circle one): (L eye) R eye Abdomen

Species: Baboons Room Tested: [redacted]

Test Reaction Grades	
Reaction Grade	Description of Changes
0----------Neg	No Reaction
1---------- Neg	Redness associated with the injection
2---------- Neg	Erythema-no swelling, serious bruising or reaction
3---------- Suspect	Minimal swelling, w/ or w/o erythema
4---------- Pos	Obvious swelling, drooping eyelid. Some degree of erythema
5---------- Pos	Swelling &/or necrosis eyelid closed

			Date: 23 Jan 21		Date: 24 Jan 21		Date: 25 Jan 21	
Animal ID	Animal Name	Wt (kg)	24hr	Initials	48hr	Initials	72hr	Initials
26741	Tara	15.5	0	[redacted]	0	[redacted]	0	[redacted]
26876	Jemma	14.0	0		0		0	
[redacted]								
28768	Juju	15.1	0		0		0	
[redacted]								

This record is authorized by law (7 USC 2131-2159). Failure to maintain this record can result in a suspension or revocation of license and/or imprisonment for not more than 1 year, or a fine of not more than $ 1,000, or both. [Form approved OMB NO. 0579-0036

U.S. Department of Agriculture Animal and Plant Health Inspection Service

RECORD OF ACQUISITION, DISPOSITION OR TRANSPORT OF ANIMALS (other than Dogs and Cats)

SALE__X__EXCHANGE OR TRANSFER_____DONATION_____

1. Invoice No.

2. Page 1 of _1__

3. Date of Dispostion 10/19/2011

INSTRUCTIONS: Complete applicable items 1 through 13. Original and one copy to accompany animals. When delivery is made - Items 14 through 20 must be completed. Original retained by Buyer (Receiver) and copy returned to Dealer (Seller or Donor). Attach Continuation Sheet (APHIS FORM 7020A) as needed.

4. Dealer's License No. [redacted]

5. Seller or Donor (Name, address, zip, phone #)
Texas Biomedical Research Institute
7620 NW Loop 410
San Antonio, Texas 78227
[redacted]

6. Buyer or Receiver (Name, address, zip, phone #)
EVMS, Dept of Comparative Med
[redacted] Norfolk VA 23507
[redacted]

7. USDA license No (if any)

8. IDENTIFICATION OF ANIMALS BEING DELIVERED

Container Tag No., Crate or Pen No.	Number Animals	Previous Invoice No. (if any)	Individual Ident., Tatoos, Tag Nos. (if applicable)	Species	Age-Sex Number Young M	Age-Sex Number Young F	Age-Sex Number Adult M	Age-Sex Number Adult F	Est. Weight (kgs)	Remarks (Condition, etc.)	Receiver Use	Receiver Use
1	1		26741	PCA			1		14.12			
[redacted]												
3	1		26876	PCA			1		14.4			
[redacted]												

DELIVERY BY COMMERCIAL CARRIER

9. Delivery by: ____Buyer's Truck _____Dealer's Truck (Seller or Donor)	10. Truck License No.	11. Bill of Lading No.

12. Name, address, phone of Company or Firm	13. Name, address, phone of Truck Driver
[redacted]	

DELIVERY RECEIPT - TO BE COMPLETED BY BUYER OR RECEIVER

14. Animals Delivered were ("x" one)	____in apparent good condition _____ poor condition _____rejected	
15. Total number received		attach explanation for rejection
16. Number Dead	17. Number Alive	
18. BY (signature)	19. Title	20. Date

APHIS FORM 7020 (APR 93)

(replaces VS FORM 18-20, which is now obsolete, and APHIS Form 7020 (10-90) which may be used.)

TEXAS BIOMEDICAL RESEARCH INSTITUTE

7620 N.W. Loop 410
San Antonio, Texas 78227

Veterinary Health Certificate
Date: October 19, 2011

Consignor	Consignee
Texas Biomedical Research Institute 7620 NW Loop 410 San Antonio, Texas 78227 [redacted] Attending Veterinarian	Eastern Virginia Medical School Dept of Comparative Medicine [redacted] Norfolk, VA 23507 [redacted]

Description of Animal(s)

Sex	Weight	Tattoo/Tag Number	Species	Last TB Test	Date of Birth	
F	14.12	26741	PCA	10/11/2011	6/1/2005	
[redacted]						
F	14.4	26876	PCA	10/11/2011	6/29/2005	
[redacted]						

Certification: I hereby certify that I have this date examined the animal(s) listed above and found them, to the best of my knowledge, to be free of clinical symptoms of any infectious or communicable disease.

Statement of Acclimatization: These animals are acclimatized to a temperature of 40° F. They should not, however, be exposed for over 30 minutes.

Remarks:

[redacted]

[redacted]

[redacted]

Received Here 5/14/15

TB/PHYSICALS/WEIGHTS

ANIMAL ID/NAME: 26876 / Jemma

INVESTIGATOR: [redacted]

ENTRY PE: 01/16/13 **EXIT PE:** _____

Date	Weight (kg)	TB	PE	OTH	Comments	Initial
[redacted]						
22 Jun 21	—	—	—	X	TB / PE	
17 Jun 21	14.0			X		
20 Jul 21	14.8	X	X	X	TB, PE, dental	

Chronological Summary Sheet

Principal Investigator	Room No.	Protocol No.	Animal Number
[redacted]		21-003	26876 "Jemma"
Vendor	**DOA/DOB**	**Date of Euthanasia**	**Species/Sex**
SWFBR	10-20-11// 6-29-05		Baboon / Female

Entry Date	Treatment and Observation	Initials
17 Jun 21	P was sedated with 1.0ml ketamine IM for fetal injection under iso. Normal recovery.	[redacted]
17 Jun 21	P was sedated with 1.0ml ketamine for c-section under iso. See anesthesia sheet and surgical notes for more details.	
20 Jul 21	Animal sedated with 2.1 ml ketamine IM for PE, blood collection, and TB test. Normal recovery. + dental TB ⊖ [redacted]	

All USDA regulated species Enrichment Records are in a different location

Chronological Summary Sheet

Principal Investigator	Room No.	Protocol No.	Animal Number
[redacted]	[redacted]	21-003	26876 Jemma
Vendor	**DOA/DOB**	**Date of Euthanasia**	**Species/Sex**
SWFBR	10-20-11 / 6-29-05		Baboon ♀

Entry Date	Treatment and Observation	Initials
5-6-21	Transfer to 21-003	[redacted]
21 MAY 21	PC- Seizure	
	S: BAR (now). Report of abnormal behavior noted during am feeding. Post ictal-type behavior. Since monkey's research treatment can cause hypoglycemia thus seizures. Treated w/ juice of fruits orally. Now BAR w/ great appetite.	
	O: fruits / juice	
	A: Research protocol induces hypoglycemia. Caretaker probably saw a post seizure behavior or a mild hypoglycemic shock.	
	P: Continue to monitor / feed regularly w/ ↓ glycemic index treats.	
24 May 21	No signs of ~~further~~ further issues. Return to normal monitoring	
15 JUN 21	Found lying down on cage.	
	S: Depressed to QAR. Salivation noted. Gums are red and swollen. Hx of found down but ate and perked up.	
	O: Metoclopramide 0.3mg/kg (1.2cc) IM once.	
	A: I think Jemma is nauseous probably dizzy 2° to CGS treatment affecting blood glucose.	
	P: Continue monitoring. Offer nutritious treats frequently. PI to consider D/C the CGS Tx	
16 JUN 21	Found unresponsive by tech @ 6AM. By the time I arrived and since she ate some apples she was up & QAR.	

All USDA regulated species Enrichment Records are in a different location

Chronological Summary Sheet

Principal Investigator	Room No.	Protocol No.	Animal Number
[redacted]	[redacted]	18-006	26876 / Jemma
Vendor	**DOA/DOB**	**Date of Euthanasia**	**Species/Sex**
SWFBR	10-20-11 / 6-29-05		Baboon / F

Entry Date	Treatment and Observation	Initials
[redacted]		
22 Jan 21	Animal sedated with 2.6 ml Ketamine IM for PE & TB test. Normal recovery. TB results came back negative.	

"All USDA regulated species Enrichment Records are in a different location"

NR = Normal Recovery

MASTER PROBLEM/EVENTS

Animal ID/Name:	26876 / Jemma	Investigator:	[redacted]

Date	Clinical Call or Event	Initials	Date of Resolution	Initials
22Jan21	TB / PE	[redacted]	25 Jan 21	[redacted]
21 MAY 21	~~Poss~~ Seizure - possibly ↓ sugar		21 may 21	
15 Jun 21	found down / salivation			
17 Jun 21	c-section			
20 JUN 21	TB, PE, dental		28 JUN 21	

POST OPERATIVE EVALUATION FORM
Species: __Baboon-Jemma____

Date of Operation:_6/17/21___________ **Protocol #:** 21-003

Pre-Operative Weight:_____________ **Animal Id** 26876- Jemma

Pain/Distress Assessment Scoring:
0= *No signs of pain or distress* (BAR with normal defecation and urination)
1= *Minimal signs of pain or distress* (BAR to QAR, subtle behavioral/physiological changes i.e. decreased defecation, reaction upon palpation of site, guarding site)
2= *Moderate signs of pain or distress* (BAR, QAR to recumbent or other behavioral changes associated with pain or distress i.e. slow movement, guarding of incision, vocalization/aggression. There may swelling/redness/discharge at site)
3= *Marked sings of pain or distress* (Recumbent, vocal, decreased defecation, urination and appetite. +/-dehiscence of incision)
4= Agonal, moribound or comatose/unresponsive
Anything above a "0" requires reassessment of analgesic regimen and contacting the attending veterinarian

Post -op Date	6-17-21		6-18-21		6-19-21		6-20-21		6-21-21		6-22-21		6-23-21		6-24-21					
Time of Day		9pm	9:30 AM	5:20 PM	8:15 AM	345p	8:20 AM	4:30 AM	10Am				1230p	11:00AM				350pm				
Active (Bar/Qar)		QAR	QAR	QAR	QAR	BAR	BAR	BAR	BAR				BAR	BAR				BAR				
Inquisitive		N	N	Y	Y	Y	Y	Y	Y				Y	Y				Y				
Urination		Y	N	Y	Y	Y	Y	Y	Y				Y	Y				Y				
Feces		N	Y	Y	Y	N	Y	Y	Y				Y	Y				Y				
Evidence of Eating		N	N	N	Y	Y	Y	Y	Y				Y	Y				Y				
Evidence of Drinking		N	Y	Y	Y	Y	Y	Y	Y				Y	Y				Y				
Gait/Posture NR		N	N	N	N	Y	Y	Y	Y				Y	Y				Y				
Incision Edge(s) Red		N	N	N	N	N	N	N	N				N	N				N				
Sutures Intact		Y	Y	Y	Y	Y	Y	Y	Y				Y	Y				Y				
Swelling present		N	N	N	N	N	N	N	N				N	N				N				
Exudate from incision		N	N	N	N	N	N	N	N				N	N				N				
Pain Score (0-4) **		2	1	1	1	1	0	0	0				0	0				0				
Medication: Banamine **Concentration Mg/ml:** 50mg/ml **Dosage cc:** 2mg/KG		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]									/				
Medication: Baytril **Concentration Mg/ml** 100mg/ml **Dosage in cc:** 5mg/KG		on Table	[redacted]	/	[redacted]	/	[redacted]	/	[redacted]									/				
Comments		Sx-5:58pm	0.3ml midazolam given IM per AV @ 10am	0.5ml midazolam given IM per AV @ 5:21pm		Looks Better			Healing well				Active					Looks Good				
Initials	[redacted]																				
CompMed review																					

Created 9-2015; Revised 6-2016

EVMS CompMed
ANIMAL MEDICAL RECORD OPERATIVE REPORT

General Information

Animal Id: 26876 Jemma TCGS E2 FI 6hrslater D155

Date/Time Field: June 17, 2021

Investigator: [redacted]

Protocol #: 21-003

Sex: f

Species/breed/strain: Baboon

Name(s) of Surgeon(s) and Assistant(s) [Personnel listed must have completed appropriate training and must be listed on the approved IACUC protocol]

[redacted]

Name (s) of Anesthesiologist

[redacted]

Surgery/Procedure description

[Description must be sufficiently detailed to facilitate examination of the animal by the veterinarian or designee]

Term CGS pregnancy with fetal injection of 10ug estradiol + estradiol benzoate On June 17th, animal was sedated with ketamine (IM) at 11:30am The fetus was located using ultrasound. 10ug of estradiol and estradiol benzoate (0.5ml) was injected into the shoulder. The mother recovered and was returned to her cage. 6hrs later, animal was sedated and intubated. Isoflurane was used during the surgery. A vertical incision was made to the abdomen and the uterus was externalized. Blood samples were taken from uterine veins. The uterus was opened and the fetus was removed. Meconium was present possibly from stress of injection, fetus was breathing. Blood samples were taken from the umbilical cord. The cord clamped and fatal plus delivered via umb V. The cord was cut once fetus was euthanized. Tissues were taken from the fetus. The placenta was delivered and appeared normal. The uterus flushed with gentamicin and oxytocin in sterile saline to assist in blood loss. Using 2-0PDS suture, the uterus was closed with simple interrupted knots. The peritoneum/fascia was closed using 2-0 PDS and a cruciate suture pattern. The fascia was then closed with a running suture line. Finally the skin was closed using 3-0 PDS subcuticular.
Baytril was prescribed to prevent infection from the meconium. Iron dextran was given as outlined in the protocol.

Description of intra-operative complications, if applicable (or comments)

No complications regarding the procedure. Notes: No major adhesions or abnormalities within the abdomen. No major or concerning issues. Animal was slow to recover from anesthesia and was pale. Solumedrol was given IV per AV order. Post-surgical analgesia was given on the table after the fetus was removed and followed as outlined in the approved protocol.

Medications administered relevant to intra-operative complications &/or emergencies
(Please list Drug name, Dose and Route plus any applicable comments)

Gentamicin 20ml in saline flush, oxytocin 1ml in saline flush, Baytril 0.9ml IM,, Solumedrol 2.0ml IV

Signature Field [redacted]

EVMS Comparative Medicine Division
NHP Surgery/Anesthesia Form

Date: 6-17-21 Animal ID: 26876 "Jemma Weight (Kg): 14.0 Protocol: 21-003
Procedure/Surgery: C-section Current wgt: Investigator: [redacted]
Surgeon: [redacted]
Anesthetist: [redacted]

Presurgical Treatment	Dose	Amt.	Route	Time	Init.

Induction	mg/kg	calc (ml)	Amt.	Route	Time	Init.
Ketamine	10	1.4	1.6	IM	5:30	[redacted]
Glycopyrolate	0.006	0.42				
Ketamine/Xylazine	[7 / 0.6]					

Endotracheal Tube			T Intub.	T Extub
			5:52	8:02
Anesthesia	Type		T Start	T End
	Isoflurane		6:00	7:46
Fluids	Type	ml/hour *	T Start	T End
	NaCl	5.6	6:01	7:48
Procedure/Surgery			T Start	T End
			6:24	7:48

* maintenance rate of 2 ml/kg/hr

Comments: 5.0 ETT
10% dextrose saline solution IV [redacted]
0.7ml banamine IM @ 7:06
0.9ml buprenex IM @ 7:27
2.0ml solu-medrol IV @ 7:43 [redacted]
1.0ml iron IM @ 7:48 [redacted]

Time	HR	SpO_2	CO_2%	RR	Iso%	BP Sys/Dia	MAP	Temp	Treatments or comments	Time	Init
6:03	150	99	4.5	25	2.0	72/42	48	98.8			[redacted]
6:15	136	99	5.0	20	2.0	57/32	44	98.7			
6:22	131	99	4.8	23	2.0	68/31	47	98.3	Begin SX	6:24	
6:28	130	99	5.7	22	2.0	76/21	39	98.3			
6:37	128	99	5.7	23	2.0	68/28	41	98.2			
6:42	128	99	5.8	23	2.0	75/57	52	98.2	Begin suture	6:52	
6:55	128	99	5.0	25	2.0	–	–	98.1	Betadine to recal		
7:08	127	99	5.1	24	2.0	58/20	41	98.1			
7:15	125	99	5.3	24	2.0	55/29	39	98.1			
7:27	120	99	5.7	21	2.0	54/34	40	97.9			
7:35	118	99	5.5	21	2.0	42/15	23	97.8	Begin skin is @ 1%		
7:44	121	99	4.9	25	0.5	–	–	97.7	ISO @ 0.5%		
7:48	130	99	4.9	26	–	71/42	57	97.7	ISO off	7:46	
8:02	–	–	–	–	–	–	–	–	chewing/stinking RTC		
8:12	–	–	–	–	–	–	–	–	Bitby alert	9:00	

Postsurgical Treatment	Dose	Amt.	Route	Time	Init.

[redacted] 6/17/21

Emergency Drugs	mg/kg	calc'd	Amt.	route	time	initials	Comments
Atropine	0.02	0.7					max 2mg/kg ↑ Heart Rate
Doxapram	2	1.4					Stimulates Respiration
Epinephrine 1:100	0.2	2.8					0.1-0.5mg/kg Improves breathing, Stimulates Heart, ↑ BP
Glycopyrrolate	0.004	0.28					
Lidocaine	1	0.14					max 3mg/kg
Prednisolone Sodi	10	7					
Phenylephrine	n/a	0.2 mg		IV			do not give more than q10 min - think of a continuous drip.

ANTECH

800-872-1001

Patient Info:
Name: JEMMA
Chart No: 26876
Owner: [redacted]
Doctor: [redacted]

Species: Primate
Breed: Baboon
Age: 16Years
Sex: F

Hospital:
Eastern VA Med ATT [redacted]
[redacted]
Norfolk, VA 23507

Antech ID: [redacted]

Lab:
ANTECH Diagnostics
3675 Concorde Pkwy
Chantilly, VA 20151
Reported: 07/22/21 01:30 AM
Received: 07/21/21

Accession No.	Doctor	Owner	Pet Name
RO			JEMMA

Test	Results	Reference Range	L / Normal / H
Superchem			
Total Protein	5.7	5.0-7.0 g/dL	
Albumin	**3.4**	**2.4-3.3 g/dL**	**HIGH**
Globulin	2.3	2.0-4.0 g/dL	
A/G Ratio	**1.5**	**0.5-1.0**	**HIGH**
AST (SGOT)	23	10-100 IU/L	
ALT (SGPT)	28	10-100 IU/L	
Alk Phosphatase	142	100-250 IU/L	
GGTP	**32**	**1-25 IU/L**	**HIGH**
Result Verified			
Total Billrubin	0.2	0.0-1.0 mg/dL	
Urea Nitrogen	18	10-22 mg/dL	
Creatinine	0.8	0.1-2.0 mg/dL	
BUN/Creatinine Ratio	23		
Phosphorus	4.3	3.0-5.5 mg/dL	
Glucose	73	70-120 mg/dL	
Calcium	8.4	7.2-11.5 mg/dL	
Magnesium	1.4	mEq/L	
Sodium	**131**	**138-150 mEq/L**	**LOW**
Potassium	3.6	3.5-5.5 mEq/L	
NA/K RATIO	36		
Chloride	**94**	**109-115 mEq/L**	**LOW**
Cholesterol	147	100-200 mg/dL	
Triglycerides	**27**	**28-109 mg/dL**	**LOW**
Amylase	**182**	**1000-2500 IU/L**	**LOW**
PrecisionPSL™	15	U/L	
CPK	388	100-400 IU/L	
Complete Blood Count			
WBC	**3.9**	**8.0-16.0 10^3/µL**	**LOW**
RBC	**4.3**	**5.50-8.50 10^6/µL**	**LOW**
Hemoglobin	**10.3**	**14.5-17.0 g/dL**	**LOW**
HCT	**33**	**38.0-50.0 %**	**LOW**
MCV	77	65-80 fL	
MCH	23.9	23.0-26.0 pg	
MCHC	**31**	**32.0-35.0 g/dL**	**LOW**
Blood Parasites	None Seen		
RBC Comment			
RBC Morphology Normal			
Platelet Count	115	10^3/µL	
Platelet count reflects the minimum number due to platelet clumping.			
Platelet EST	Adequate		

Differential	Absolute	%		
Neutrophils	**2184**	**56**	**4800-12000 /µL**	**LOW**
Bands		0		
Lymphocytes	**1560**	**40**	**1600-4800 /µL**	**LOW**
Monocytes	156	4	0-480 /µL	
Eosinophils	**0**	**0**	**80-800 /µL**	**LOW**
Basophils	0	0	/µL	

Comment
Blood smear reviewed by technologist.

APPROVED
By [redacted] at 10:27 am, Jul 22, 2021

FINAL

For

TB Test Worksheet

Date of Test: 20 Jul 21 Test Site (circle one): L eye **R eye** Abdomen

Species: Baboons Room Tested: [redacted]

Test Reaction Grades	
Reaction Grade	Description of Changes
0---------- Neg	No Reaction
1---------- Neg	Bruise-associated with the injection
2---------- Neg	Erythema-no swelling, serious bruising or reaction
3---------- Suspect	Minimal swelling, w/ or w/o erythema
4---------- Pos	Obvious swelling, drooping eyelid. Some degree of erythema
5---------- Pos	Swelling &/or necrosis eyelid closed

			Date: 21 Jul 21		Date: 22 Jul 21		Date: 23 Jul 21	
Animal ID	Animal Name	Wt (kg)	24hr	Initials	48hr	Initials	72hr	Initials
[redacted]								
26876	Jemma		0		0		0	
28768	Juju		0		0		0	
							[redacted]	

[redacted] 22 Aug 21

Physical Exam

Date: 20 Jul 2021

Animal Name: Jemma

Animal ID: 28768

Species: Baboon

Sex: M / (F)

Investigator: [redacted]

Initial Ketamine: 1.4 cc

+ Add. Used: 0.7 cc + 0.1ml xylazine (100mg/ml)

= Total Ketamine: 2.1 cc

Animal Weight: 14.8 kg

Physical Type: ☐ Entry ☐ Exit ☐ Annual ☑ Biannual

Physical Findings

Overall

Temp: 98.8 deg F	Pulse: _____ bpm	Resp: 20 br./min

E E N T:	☑ Normal	☐ Abnormal
Head:	☑ Normal	☐ Abnormal
Heart/Lungs:	☑ Normal	☐ Abnormal
Abdomen:	☑ Normal	☑ Abnormal
Lymph Nodes:	☐ Normal	☐ Abnormal
Arms/Hands:	☑ Normal	☐ Abnormal
Legs/Feet:	☑ Normal	☐ Abnormal
Genitals:	☑ Normal	☐ Abnormal
Coat/Tail:	☐ Normal	☐ Abnormal

Dentition
(Grade 0-5): Tartar: 3/5 Gingivitis: 3-4/5

Notes/Missing or Abnormal Teeth:

Dental Needed? ☒ Yes — Done today

Notes/Comments:

TB TEST ADMINISTERED ☒ R

- fractured w/ exposed nerve (R) upper canine - Schedule for extraction
- missing (R) hand thumbs
- Surgery scar

Laboratory Workup: ☐ None ☒ CBC/Chemistry ☐ Fecal

Lab. Used: Antech ☐ SRS/SIV ☐ Herpes B ☐ FILO

[redacted] 7/22/21

Performed by: [redacted]

Physical Exam

NOTES ON Dental prophy exams

Date: 20/Jul/2021

Animal Name: EMMA

Animal ID: 28768

Species: BABOON

Sex: M / (F)

Investigator: [redacted]

Initial Ketamine: ____ cc

+ Add. Used: ____ cc

= Total Ketamine: ____ cc

N/A already sedated

Animal Weight: 14.8 kg

Physical Type: ☐ Entry ☐ Exit ☒ Annual ☐ Biannual

Physical Findings

Temp: ____ deg F | **Pulse:** ____ bpm | **Resp:** ____ br./min

E E N T:	☐ Normal	☐ Abnormal
Head:	☐ Normal	☐ Abnormal
Heart/Lungs:	☐ Normal	☐ Abnormal
Abdomen:	☐ Normal	☐ Abnormal
Lymph Nodes:	☐ Normal	☐ Abnormal
Arms/Hands:	☐ Normal	☒ Abnormal
Legs/Feet:	☐ Normal	☐ Abnormal
Genitals:	☐ Normal	☐ Abnormal
Coat/Tail:	☐ Normal	☐ Abnormal

N/A: NOTES for DENTAL: [redacted]

Dentition
(Grade 0-5): **Tartar:** 3/5 **Gingivitis:** 3-5/5

Notes/Missing or Abnormal Teeth:

Dental Needed? ☐ Yes

Overall Notes/Comments:
- Upon further exam and after Dentine prophylaxis, extraction of (R) upper canine is NOT needed.
- Dental prophy performed ✓

Laboratory Workup: ☒ None ☐ CBC/Chemistry ☐ Fecal

Lab. Used: ____ ☐ SRS/SIV ☐ Herpes B ☐ FILO

Performed by: [redacted]

TB Test Worksheet

Date of Test: 22 Jan 21 Test Site (circle one): (L eye) R eye Abdomen

Species: Baboons Room Tested: [redacted]

Test Reaction Grades	
Reaction Grade	Description or Changes
0----------Neg	No Reaction
1---------- Neg	Bruise associated with the injection
2---------- Neg	Erythema-no swelling, serious bruising or reaction
3---------- Suspect	Minimal swelling, w/ or w/o erythema
4---------- Pos	Obvious swelling, drooping eyelid. Some degree of erythema
5---------- Pos	Swelling &/or necrosis eyelid closed

			Date: 23 Jan 21		Date: 24 Jan 21		Date: 25 Jan 21	
Animal ID	Animal Name	Wt (kg)	24hr	Initials	48hr	Initials	72hr	Initials
26741	Tara	15.5	0	[redacted]	0	[redacted]	0	[redacted]
26876	Jemma	14.0	0		0		0	
[redacted]								
28768	Juju	15.1	0		0		0	
[redacted]								
							[redacted]	

1/25/21

Physical Exam

Date: 22 Jan 2021

Animal Name: Jemma

Animal ID: 26876

Species: Baboon

Sex: M (F)

Investigator: [redacted]

Initial Ketamine: 1.4 cc

+ Add. Used: +0.1 +0.5 cc

= Total Ketamine: (1.3) cc

Animal Weight: wasn't recorded [redacted] kg

Physical Type: ☐ Entry ☐ Exit ☐ Annual X Biannual

Physical Findings

Overall — **Temp:** 101.0 deg F | **Pulse:** 124 bpm | **Resp:** 28 br./min

E E N T:	☑ Normal	☐ Abnormal
Head:	☑ Normal	☐ Abnormal
Heart/Lungs:	☐ Normal	☐ Abnormal
Abdomen:	☑ Normal	☐ Abnormal
Lymph Nodes:	☑ Normal	☐ Abnormal
Arms/Hands:	☐ Normal	☒ Abnormal
Legs/Feet:	☑ Normal	☐ Abnormal
Genitals:	☑ Normal	☐ Abnormal
Coat/Tail:	☐ Normal	☒ Abnormal

Dentition (Grade 0-5): Tartar: 3 Gingivitis: 3

Notes/Missing or Abnormal Teeth: [redacted]

Dental Needed? ☒ Yes 11/25/21

Notes/Comments:

TB TEST ADMINISTERED ☑

Alopecia (R) shoulder

Missing joints on (L) hand (All four digits)

Laboratory Workup: ☒ None ☐ CBC/Chemistry ☐ Fecal

Lab. Used: ✓ ☐ SRS/SIV ☐ Herpes B ☐ FILO

Performed by: [redacted]