

**PROTOCOL FOR ANIMAL USE AND CARE**Email to: [campusvet@ucdavis.edu](mailto:campusvet@ucdavis.edu)**CNPRC**

EH&amp;S USE ONLY

**PROTOCOL: 10776**  
**EXPIRES: 08/13/04**

Investigator		Contact	
Last Name:		Last Name:	
First:		First:	
Middle:		Middle:	
email:		email:	
Department:		Department:	
Phone / Fax:		Phone / Fax:	
After hrs. #:		After hrs. #:	

Species (common names):	Number:	Source:
Rhesus macaque	12	CNPRC

**Project Title** Effects of blockade of VEGF action on endometrial development and differentiation.

Overnight housing location:	CNPRC	Day use only :	CNPRC
Animals will be maintained by:	<input checked="" type="checkbox"/> Vivarium <input type="checkbox"/> Investigator (If investigator maintained, attach husbandry SOP's.)		

**Procedures:** Provide a one or two sentence layman's description of the procedures employed on the animals in this project. This information will help the animal care staff understand any conditions they may encounter while caring for your animals.

All macaques will be ovariectomized and will be treated with estradiol (E<sub>2</sub>) and progesterone (P) implants to induce an artificial menstrual cycle. The endometrium will be collected by hysterectomy or by endometrial biopsy at the end of the second artificial cycle following different treatments as described below.

**Special Husbandry Requirements:** Describe any special requirements your animals have with respect to **food, water, temperature, humidity, light cycles, caging type, bedding**, or any other conditions of husbandry.

No special husbandry required.

Other instructions for animal care staff: (check applicable entries)

Sick Animals	Dead Animals	Pest Control
<input checked="" type="checkbox"/> Call Investigator	<input checked="" type="checkbox"/> Call Investigator	<input type="checkbox"/> Call Investigator
<input checked="" type="checkbox"/> Clinician to treat	<input checked="" type="checkbox"/> Save for Investigator	<input checked="" type="checkbox"/> OK to use pesticides
<input type="checkbox"/> Terminate	<input type="checkbox"/> Bag for disposal	<input type="checkbox"/> No Pesticides in animal area
<input checked="" type="checkbox"/> Necropsy	<input checked="" type="checkbox"/> Necropsy	

**Hazardous Materials** (only if in the animal room):

Infectious Agents?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Radioisotopes?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Chemical Carcinogens?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Toxic Chemicals?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	

Funding source:	Mellon Foundation	Previously approved?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No
Is the project already funded?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Previous protocol number (if any):	

What Veterinarian or veterinary clinic will provide care for your animals? (check one)

<input type="checkbox"/>	Lab Animal Health Clinic ( 2-0514 )	<input checked="" type="checkbox"/>	California Primate Research Center ( 2-0447 )
<input type="checkbox"/>	VMTH Large Animal Field Service ( 2-0292 )	<input type="checkbox"/>	Another Veterinarian

If you checked "Another Veterinarian", please provide:

Veterinarian:		Address:	
Day phone:			
Emergency phone:		Email:	

*If your veterinarian is not affiliated with one of the three service units listed above, please contact the campus veterinarian, 2-2357 (email [pcstillman@ucdavis.edu](mailto:pcstillman@ucdavis.edu)) for current information about training and record keeping requirements.*

### Summary of Procedures:

a) Briefly describe the **overall intent** of the study. Include in your description a statement of your hypothesis, the objectives and significance of the study. Your target audience is a faculty member from a discipline unrelated to yours. Do not use jargon.

This proposal aims to gain new knowledge regarding mechanisms of endometrial vascular development and its role in endometrial growth and differentiation of primate endometrium during the menstrual cycle. We have previously ( , 2002) demonstrated a dramatic estrogen-dependent peak in vascular proliferation in the mid-proliferative phase endometrium (day 8-10 after Progesterone withdrawal), which coincided with a peak in **vascular endothelial growth factor (VEGF)** expression in the endometrial stroma. In a recent collaborative study with Regeneron Pharmaceuticals, we have shown that blockade of VEGF action during postmenstrual repair phase by VEGF-trap (a soluble VEGF blocker) can completely block the midproliferative vascular development in the upper zones of endometrium. These results indicate that VEGF plays an essential role in the midproliferative phase vascular development in primate endometrium. We believe that blockade of the VEGF-dependent vascular development that occur in the upper zones of endometrium during the midproliferative phase would render the upper endometrium avascular throughout the menstrual cycle leading to inhibition of normal endometrial development during the proliferative phase and P-induced transformation of the endometrium during the secretory phase. Also, we believe that blockade of VEGF action during the later stages of the cycle (after postmenstrual repair phase) may not significantly inhibit endometrial vascular development and overall endometrial development and differentiation.

To test these hypothesis, we propose the following experiments as described below to examine the effects of early-proliferative (postmenstrual repair phase) vs. late-proliferative phase administration of VEGF-trap on endometrial vascular development and overall endometrial development and differentiation at the end of the cycle.

## b) Procedures employed in this project:

Please check the appropriate boxes if any of these procedures will be employed in your project:

- |   |  |  |
|---|--|--|
| <input type="checkbox"/> Monoclonal Antibody Production **                  | <input type="checkbox"/> Food or water restriction               | <input type="checkbox"/> Special diets; food or water treatment.   |
| <input type="checkbox"/> Polyclonal Antibody Production **                  | <input type="checkbox"/> Non-recovery surgical procedures        | <input type="checkbox"/> Induced illness, intoxication, or disease |
| <input type="checkbox"/> LD 50 or ID50 studies.                             | <input checked="" type="checkbox"/> Survival surgical procedures | <input type="checkbox"/> Death as an endpoint (see i below)        |
| <input checked="" type="checkbox"/> catheters, blood collection, intubation | <input checked="" type="checkbox"/> Multiple survival surgery    | <input type="checkbox"/> Trapping, banding or marking wild animals |
| <input type="checkbox"/> Prolonged restraint. (8 hrs+)                      | <input type="checkbox"/> Behavioral modification.                | <input type="checkbox"/>   |
| <input checked="" type="checkbox"/> Fasting prior to a procedure.           | <input type="checkbox"/> Aversive conditioning.                  | <input type="checkbox"/>   |

\*\* If this protocol only describes antibody production, you may use the attached antibody production page in lieu of completing section c below.

c) Describe the use of animals in your project in detail, with special reference to any of procedures checked above. Include any physical, chemical or biological agents that may be administered. List each study group, and describe all the specific procedures that will be performed on each animal in each study group. Use terminology that will be understood by individuals outside your field of expertise. (Note: This cell will expand to whatever length you require. You may make this section as long as you wish, but try to be concise. Some projects may require one or two pages.)

**Experiment 1. Effects of early-proliferative phase administration of VEGF-trap on vascular development and P-induced transformation of the endometrium at the end of the cycle:** Six ovariectomized adult rhesus monkeys will be used for this study, three controls (group 1) and three VEGF-trap treated (group 2) animals (n=3). All animals will be anesthetized with ketamine (10 mg/kg), intubated and placed on isoflurane anesthesia. The animals will be given atropine (0.04 mg/kg) and receive a standard ovariectomy by laparotomy or laparoscopy by CNPRC surgical staff. Following surgical recovery all macaques will be treated sequentially with estradiol (E<sub>2</sub>) and progesterone (P) implants to create artificial menstrual cycles. All implants (both E<sub>2</sub> and P) will be inserted and removed from the animals at various timepoints (described below) while animals are briefly immobilized with ketamine (10 mg/kg) for each procedure.

Each macaques will receive **one** s.c. (subcutaneous) implant of 3-cm Silastic capsule of E<sub>2</sub> by a 1cm skin incision in the suprascapular region to stimulate development of an artificial proliferative phase endometrium. After 14 days, **one** 6-cm Silastic capsule of P will be implanted s.c. **by a 1cm skin incision in the suprascapular region (opposite side of E<sub>2</sub> implant)**, and both implants will remain in place for 14 days to stimulate an artificial secretory phase endometrium. Then at the end of the artificial cycle, the P implants will be removed by 1 cm cutaneous (skin) incision to induce menstruation while the E<sub>2</sub> implants remain in place. Three animals will be treated with VEGF-trap at a dose rate of 12.5 mg/kg (diluted in 10ml of phosphate buffered salt solution (PBS)) intravenously on days 2, 4, and 6 after P withdrawal, and the control animals (three) will be treated similarly with the vehicle (PBS plus human IgG Fc fragment, 12.5 mg/kg). On day 14 after P withdrawal, the P implants will be inserted again and will remain in place for 14 days to mimic secretory transformation of the endometrium. At the end of the cycle i.e. after 14 days of P treatment, the endometrium will be collected either by euthanizing the animals or by hysterectomy or by a full-thickness endometrial biopsy. If hysterectomy or full-thickness endometrial biopsy is used, the animals will be anesthetized with ketamine (10 mg/kg), intubated and placed on isoflurane anesthesia. The animals will be given atropine (0.04 mg/kg) and receive a standard hysterectomy/biopsy by CNPRC surgical staff. The animals will be monitored for presence of vaginal bleeding by vaginal swabbing after P withdrawal. Both the control and trap-treated animals will be administered with intravenous infusion of 10 ml of Br-dU (5-bromo-2'-deoxyUridine @ 10mg/ml of PBS) at three time points, starting 24, 16, and 2 hours before tissue collection. A total of five peripheral venous blood samples (5 ml each) will be collected from each animal during the entire experiment (first collection, one day after E<sub>2</sub> implant; second collection, one day after P implant; third collection, one day after P implant removal; fourth collection during insertion of P implant (second cycle); and fifth collection during tissue (endometrium) collection). Blood samples will be collected while animals are cage restrained for first three time-points, and

during P implant and hysterectomy/biopsy surgery for fourth and fifth blood collection. If the endometrium will be collected by hysterectomy/biopsy, the animals will be used for another IACUC approved protocol or will be released for use by other investigators.

**Experiment 2. Effects of late-proliferative phase administration of VEGF-trap on vascular development and P-induced transformation of the endometrium at the end of the cycle:**

Six ovariectomized adult rhesus monkeys will also be used for this study, three controls (group 3) and three VEGF-trap (group 4) treated animals (n=3). This experiment will be performed identical to the above experiment (experiment-1) except that the vehicle or VEGF-trap will be administered on days 10, 12, and 14 (late-proliferative phase) after P withdrawal.

**Note:** (i) VEGF-Trap is a very potent inhibitor of VEGF action, and systemic administration of this compound at very high doses (25 mg/kg/d, sc) has shown no signs of toxic effects in several species including marmoset, rhesus, stump-tail, and cynomolgus monkeys.

(ii) Br-dU is not a radioisotope. At the Oregon National Primate Research Center, we (Dr. ) and others have used the above doses of Br-dU in rhesus monkeys without any toxic effects ( , 2002).

**d) Study Groups and Numbers:** Define, in the form of a table, the numbers of animals to be used in each experimental group described above. The table may be presented on a separate page as an attachment to this protocol if you prefer. The Normal format should be three columns: Study Group, Procedure, Number of animals. The number of rows should follow from the number of study groups; **you may add as many rows as you require**. The chart must fully account for the number of animals you intend to use under this protocol. Assign each group to an invasiveness category according to the chart below.

Group	Procedures / Drugs	Number of Animals	Category
1	Intravenous vehicle infusion (control, experiment #1) on days 2, 4, and 6 after P withdrawal.	3	3
2	Intravenous VEGF-trap infusion on days 2, 4, and 6 after P withdrawal.	3	3
3	Intravenous vehicle infusion (control, experiment #2) on days 10, 12, and 14 after P withdrawal.	3	3
4	Intravenous VEGF-trap infusion on days 10, 12, and 14 after P withdrawal.	3	3

## Categories of invasiveness

Category	Description
1	Little or no discomfort or stress <b>Examples:</b> domestic flocks or herds being maintained in simulated or actual commercial production management systems; the short-term and skillful restraint of animals for purposes of observation or physical examination; blood sampling; injection of material in amounts that will not cause adverse reactions by the following routes: intravenous, subcutaneous, intramuscular, intraperitoneal, or oral.
2	Minor stress or pain of short duration <b>Examples:</b> cannulation or catheterization of blood vessels or body cavities under anesthesia; minor surgical procedures under anesthesia, such as biopsies or laparoscopy; short periods of restraint beyond that required for simple observation or examination, but consistent with minimal distress
3	Moderate to severe distress <b>Examples:</b> major surgical procedures conducted under general anesthesia, with subsequent recovery; prolonged (several hours or more) periods of physical restraint; induction of behavioral stresses such as maternal deprivation
4	Severe pain near, at or above the pain tolerance threshold <b>Examples:</b> exposure to noxious stimuli or agents whose effects are unknown; exposure to drugs, chemicals, or infectious agents at levels that markedly impair physiological systems and which cause death, severe pain, or extreme distress; Surgical experiments which have a high degree of invasiveness.

Further descriptions of these categories are included in the instructions following this document.

e) **Rationale for species and numbers:** How did you determine that 1) the species choice was appropriate and 2) the number of animals in each study groups was the minimum number necessary to achieve sound scientific results?

The aim of this study is to gain new knowledge regarding mechanisms of endometrial vascular development and its role in endometrial growth and differentiation of primate endometrium during the menstrual cycle. Rhesus macaques are essential for this work because they menstruate identically to women. Endometrial physiology including regulation by steroid hormones and menstruation, have been studied more intensively in rhesus macaques than any other nonhuman primate species. The rhesus macaque endometrium differentiates into morphological zones that are very similar to those of women. Moreover, we would like to compare the results of this study with our previous findings in the rhesus macaque model. The procedures and the number of animals (n=3) proposed in this study are the minimum number of animals required for statistical validity of the results.

f) **Surgery:** If the project involves survival surgery, where will the surgery be conducted?

Building:  Room:

Who will be the surgeon?

g) **Anesthetics, Analgesics, Tranquilizers, Neuromuscular blocking agents:**

Post procedural analgesics should be given whenever there is possibility of pain or discomfort that is more than slight or momentary. If postoperative analgesics are not to be given, justify the practice under part (i) below.

Provide the following information about any of these drugs that you intend to use in this project.

Species	Drug	Dose (mg/kg)	Route	When and how often will it be given?
Rhesus	Ketamine	10	IM	For immobilization (once per ovariectomy, implant placement, implant removal and hysterectomy)
	Isoflurane	Inhaled	To eff	Surgery (ovariectomy surgery and hysterectomy/biopsy surgery)
	Oxymorphone	.15	IM	Two days post op (one regimen per two surgeries 2-3X/day)

h) **Neuromuscular blocking agents** can conceal inadequate anesthesia and therefore require special justification. If you are using a neuromuscular blocking agent, please complete the following:

Why do you need to use a neuromuscular blocking agent?

What physiologic parameters are monitored during the procedure to assess adequacy of anesthesia?

Under what circumstances will incremental doses of anesthetics-analgesics be administered?

**i) Adverse effects:**

Describe any potential adverse effects of the experiment on the animals (such as pain, discomfort; reduced growth, fever, anemia, neurological deficits; behavioral abnormalities or other clinical symptoms of acute or chronic distress or nutritional deficiency)

We do not anticipate any potential adverse effects of the experiment except minor stress or pain after hysterectomy. We also do not anticipate any complications of surgery during the experiment, as surgeries like laparotomy and hysterectomy are routinely performed by the CNPRC veterinary staffs. However, we will observe these animals for any signs of illness, particularly for any complications of surgery/biopsy and/or infection, and will notify the veterinary staff for necessary treatment.

How will the signs listed above be ameliorated or alleviated? If signs are not to be alleviated or ameliorated by means of post-operative analgesics or other means, explain why this is necessary.

Postoperative analgesics will be administered at the request and guidance of the veterinarian.

*Note: if any unanticipated adverse effects not described above do occur during the course of the study, a complete description of those effects and the steps taken to mitigate them must be submitted to the committee as an amendment to this protocol.*

Is death an endpoint in your experimental procedure?  Yes  No

*(Note: "Death as an endpoint" refers to acute toxicity testing, assessment of virulence of pathogens, neutralization tests for toxins, and other studies in which animals are not euthanized, but die as a direct result of the experimental manipulation). If death is an endpoint, explain why it is not possible to euthanize the animals at an earlier point in the study. If you can euthanize the animals at an earlier point, describe the clinical signs which will dictate that an animal will be euthanized.*

**j) Literature search for alternatives and unnecessary duplication:**

*Federal law specifically requires this section. You are required to conduct a literature search to determine that either 1) there are no alternative methodologies by which to conduct this class/lab, or 2) there are alternative methodologies, but these are not appropriate for your particular class/lab. "Alternative methodologies" refers to reduction, replacement, and refinement (the three R's) of animal use, not just animal replacement. You must also show that this use of animals is not **unnecessarily** duplicative of other studies.*

*UC Davis provides on-line access to a number of databases that can be used to search for alternatives. Visit [http://trc.ucdavis.edu/jawelsh/Databases/Databases\\_Med\\_Vet\\_Researchers.htm](http://trc.ucdavis.edu/jawelsh/Databases/Databases_Med_Vet_Researchers.htm) (email: [jawelsh@ucdavis.edu](mailto:jawelsh@ucdavis.edu)) or [http://www.vetmed.ucdavis.edu/Animal\\_Alternatives/main.htm](http://www.vetmed.ucdavis.edu/Animal_Alternatives/main.htm) (email: [mwwood@ucdavis.edu](mailto:mwwood@ucdavis.edu))*

What was the date on which you conducted this search?

07/10/03

List the databases searched or other sources consulted (there should be more than one). Include the years covered by the search.

Database Name	Years Covered	Keywords / Search Strategy
PubMed	1990-2003	Endometrium, angiogenesis, endometrial vascular development, endometrial differentiation, Er, PR, menstruation, nonhuman primate, healing, progesterone withdrawal, rhesus macaque, laparoscopy,

		laparotomy, hysterectomy, endometrial cell culture, endometriectomy, endometrial bleeding, MMPs, angiogenic growth factors etc.
ISI Web of Knowledge	1990 to 2003	Same

What were your findings with respect to alternative methodologies?

The literature searches described above provided evidence that abnormal uterine bleeding is a significant cause of suffering for women which accounts for more than half of the clinical practice in gynaecology. The study further confirmed that studies of endometrial vascular development and bleeding can only be done in an in vivo model. Steroid hormones are responsible for normal and abnormal endometrial bleeding, but the identity of locally operative factors in endometrial bleeding and healing are not known. Breakthrough bleeding is the most important cause for the discontinuation of extremely effective steroid contraceptives. Systemic or local administration of VEGF antagonists can block vascular development and wound healing. Menstruation only occurs in women and nonhuman primates. Angiogenesis in the endometrium and menstruation occur as a result of complex interaction of several cell types in the primate endometrium, even by recruiting immune cells from systemic circulation. There is also a zonal gradient in expression of different tissue factors in the endometrium from cells in the functionalis to basalis. Therefore, nonhuman primates are the only suitable experimental animal model for this work and no alternatives to the proposed research exist. There are no less painful and/or stressful alternatives methods for tissue collection, and for insertion and removal of silastic implants of estradiol and progesterone.

Has this study been previously conducted?

Yes  No

If the study has been conducted previously, explain why it is scientifically necessary to replicate the experiment.

k) **Disposition of animals:** At what point in the study, if any, will the animals be euthanized?

Euthanasia is not part of the experimental design, but will be at the discretion of a senior veterinarian.

l) **Methods of euthanasia:** Even if your study does not involve killing the animals, you should show a method that you would use in the event of unanticipated injury or illness. If anesthetic overdose is the method, show the agent, dose, and route.

Species	Method	Drug	Dose (mg/kg)	route
Rhesus	Overdose	Pentobarbital	60	IM

m) **Surplus animals:** What will you do with any animals not euthanized at the conclusion of the project?

To be returned to the CNPRC colony.



Assurances for the Humane Care and Use of Vertebrate Animals:

Principal Investigator's Statement:

I have read and agree to abide by the *UC Davis Policy and Procedure Manual* section 290-30 (Animal Use and Care). This project will be conducted in accordance with the *ILAR Guide for the Care and Use of Laboratory Animals*, and the *UC Davis Animal Welfare Assurance* on file with the US Public Health Service. (These documents are available from the Campus Veterinarian and at <http://ehs.ucdavis.edu/>). I will abide by all Federal, state and local laws and regulations dealing with the use of animals in research.

I will advise the Animal Use and Care Administrative Advisory Committee in writing of any significant changes in the procedures or personnel involved in this project.

_____ <i>Principal Investigator</i>	_____ <i>Rank / Title</i>	_____ <i>Date</i>
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Committee Use Only Below

<b>** Conditions necessary for Committee Approval:</b>
Final Disposition of this protocol: <input type="checkbox"/> Approved <input type="checkbox"/> Not Approved <input type="checkbox"/> Withdrawn by Investigator  Date of Action: ____/____/____

I verify that the Institutional Animal Care and Use Committee of the University of California, Davis, acted on this protocol as shown above.

_____ <i>Campus Veterinarian</i>	_____ <i>Date</i>
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Date: Fri, 01 Aug 2003 14:40:23 -0700  
 To: <  
 From:  
 Subject: Fwd: Re: Fwd: pre reveiw questions 10776

Date: Fri, 01 Aug 2003 14:03:36 -0700  
 To:  
 From:  
 Subject: Re: Fwd: pre reveiw questions 10776  
 Cc:

Hi,  
 Here is the 10776 protocol with additional information added from        questions.

Thanks,

At 12:13 PM 8/1/2003 -0700, you wrote:

'  
 Questions from        .

Date: Fri, 01 Aug 2003 10:29:48 -0700  
 To:  
 From: >  
 Subject: pre reveiw questions 10776  
 Cc:

Hi ,

I have received and pre reviewed the recently submitted        protocol which has been assigned accession number 10776 for future reference. I have attached a copy of the protocol for ease of making revisions. For this protocol to be considered on the Aug 14th committee agenda, please forward your revised protocol to me on or before noon, Tuesday, Aug 5th.

If you have any questions feel free to contact me.

Thank you in advance,

Protocol 10776 ( )

1. On page 1 the following boxes were left blank: overnight housing, day use and who will maintain the animals. Please complete the blank boxes.
2. In section a, you use the acronym VEGF. What does that acronym stand for? Please clarify.
3. In section c, the following questions need clarifying:
  - a. In Expt 1, paragraph 2, you mention s.c. implants of silastic capsules. How many capsules will be implanted?
  - b. After 14 days another implant will be placed, but you have not described the location for the second implant. Please expand this section to include the location of the second implant.
4. In section g, you have listed Oxymorphone to be administered two days post op. With what frequency will that be administered? SID or BID? Please clarify.
5. In section i, adverse effects, you state that you do not anticipate any potential adverse effects, but what about surgical or biopsy complications? Infections? Please expand to address potential problems.

6. In section j, the date on your literature search is listed as 10/07/03. Is this the European version of 07/10/03??  
Do you mean July 10th? Please clarify.

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