

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

EH&amp;S USE ONLY

**PROTOCOL: 10321  
EXPIRES:**

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

Species (common names):	Number:	Source:
Rhesus Macaque	24	CNPRC Colony

<b>Project Title</b>	Effect of Physical Stress on the Reproductive Cycle		
Overnight housing location::	CNPRC	Day use:	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.

Twenty-four females will be randomly assigned twice (crossover design) into four groups. Groups 1-3 will receive 1, 2 or 3 consecutive days of a single day capture/hand restraint and blood collection respectively. Group 4 will not receive any physical restraint or blood collection. Daily urine samples will be collected from all groups throughout treatment and follow-up cycles for approximately 5-6 months.

**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.

During this study, it is imperative that animals are not subjected to any additional types of stress, including but not limited to relocation, weighing, and TB testing. Since these animals will be subjected to specific stressors at specific time-points during the study, additional stressful interventions may interfere with the study.

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 checked="" type="checkbox"/> Call Investigator
<input checked="" type="checkbox"/> Clinician to treat	<input type="checkbox"/> Save for Investigator	<input 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 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:	NIESH	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):	none

**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 pctlillman@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.

The objective of this study is to determine the dose response effects of a physical stressor on the menstrual cycle. In this study, animals will be subjected to varying amounts of the same physical stress of capture and hand restraint followed by blood collection. Previous observational studies by our group in women indicate that physical stresses during the luteal-follicular transition can lead to a delay in ovulation in the next menstrual cycle ( et al., 1998). We recognize that a delay in ovulation is in itself not a health risk, but we have also shown that it can be associated with increased bone loss ( et al., 1997; et al., 2002). An increased bone loss in association with delayed ovulation has also been observed in the laboratory macaque ( , preliminary observation and the basis of funding). If the current study produces data indicating that the physical stress of capture and restraint in the luteal-follicular phase causes a delay of ovulation with associated bone loss, then this model will be used in future studies to understand the mechanism by which physical stress and/or delayed ovulation contributes to bone loss in young women.

**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 type="checkbox"/> Survival surgical procedures     | <input type="checkbox"/> Death as an endpoint (see i below)        |
| <input checked="" type="checkbox"/> catheters, blood collection, intubation | <input 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 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.)

All animals will have daily urine samples (3 cc/day/animal) collected from cage pans placed below their respective cages for approximately five to six months of collection.

In this study, twenty-four adult rhesus macaques, with normal menses bleeding patterns will be randomly assigned twice into four different groups (n=6 per group). In the first treatment assignment (first round), all animals will receive treatment starting on day 10 following ovulation (ovulation determined by a rise in urinary FSH as measured from collected urine samples). Animals in group 1 will receive a single treatment of capture and hand restraint followed by blood collection (drawn from femoral or saphenous vein; 5 cc into red-top vacutainer) on day 10 (n=1 treatment). Animals in group 2 will receive the same treatment on days 10-11 (n=2 treatments). Animals in group 3 will receive the same treatment on days 10-12 (n=3 treatments). Animals in group 4 (control group) will not receive any treatment, only daily urine samples as well as confirmation of ovulation by measurement of urinary FSH. After animals have undergone their respective treatments in the first round, urine collection will continue through the next two cycles (results and recovery cycles respectively).

Following the recovery cycle from the first round of assignments and treatments (cycle 4), all animals will be randomly assigned again, into these same treatment groups as outlined above. All animals will undergo their respective group treatment procedure (as outlined above) followed by another results cycle (cycle 5) of urine collection. After the completion of cycle 5, all animals will be returned to the CNPRC colony.

Blood samples will be analyzed for progesterone concentrations to both confirm the initiation of the stress as well as to detect any acute effect on ovarian function. Urine samples will be analyzed for estrogen and progesterone metabolites and FSH to evaluate effects of treatments on the reproductive cycle. In addition, urine samples will be analyzed for bone markers to assess bone loss.

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	One capture/hand restraint	6 (round 1) 6 (round 2)	1
2	Two captures/hand restraints	6 (round 1) 6 (round 2)	1
3	Three captures/hand restraints	6 (round 1) 6 (round 2)	1
4	No capture/no hand restraint	6 (round 1) 6 (round 2)	1

## 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 rhesus macaque has been chosen as the animal model due to its similarities to humans with respect to menstrual cycle characteristics, hormonal profiles as well as response to stress.

A sufficient number of animals are needed per group (n=6 animals per group per round) in order to account for the variability in normal reproductive and endocrine characteristics. This crossover assignment of animals into two treatment rounds will allow for us to be able to use a smaller group of animals overall, and thus determine a possible dose-response effect on the menstrual cycle from increasing amounts of physical stress.

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?

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 expect any adverse effects of the treatment on the animals. We do anticipate a stress related endocrine cycle perturbation such as a delay or prevention of ovulation that will not be permanent.

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.

Any adverse effects will be ameliorated or alleviated with analgesics 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\\_Med\\_Vet\\_Researchers.htm](http://trc.ucdavis.edu/jawelsh/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: [mwood@ucdavis.edu](mailto:mwood@ucdavis.edu))*

What was the date on which you conducted this search?

9/25/02

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
Melvyl	1988-2002	Menstrual cycle/physical stress
Biosis	1985-2002	Menstrual cycle/physical stress

What were your findings with respect to alternative methodologies?

Opportunities to study the effect of stress on the menstrual cycle are limited to humans, great apes and simian primates. Observational studies have already been completed on humans by our group, as well as other investigators, and these data are the basis for the current experimental design.

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
M. mulatta	Overdose as per CNPRC guidelines	Pentobarbital	60 mg/kg	IV

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

Return to the CNPRC colony.



