**PROTOCOL FOR ANIMAL USE AND CARE**

**CNPRC**

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Contact</th>
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<tr>
<td>Last Name:</td>
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<td>Phone / Fax:</td>
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<td>After hrs. #:</td>
<td>After hrs. #:</td>
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**Species (common names):** Cynomolgus Macaque  
**Number:** 6  
**Source:** CRPRC

**Project Title:** Modification of sperm surface proteins following transit through the macaque cervix.

**Overnight housing location:**  
**Day use:**

**Animals will be maintained by:**  
[ ] Vivarium  
[ ] 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.

Female cynomolgus monkeys will be anesthetized briefly with ketamine 2 hours post-coitus for collection of sperm via vaginal lavage and 18-24 hours post-coitus for aspiration of sperm in mucus from the cervical os and from uterine fluid via ultrasound guided aspiration.

**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 requirements will be required.

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

<table>
<thead>
<tr>
<th>Sick Animals</th>
<th>Dead Animals</th>
<th>Pest Control</th>
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</thead>
<tbody>
<tr>
<td>[ x ] Call Investigator</td>
<td>[ x ] Call Investigator</td>
<td>[ x ] Call Investigator</td>
</tr>
<tr>
<td>[ x ] Clinician to treat</td>
<td>[ ] Save for Investigator</td>
<td>[ x ] OK to use pesticides</td>
</tr>
<tr>
<td>[ ] Terminate</td>
<td>[ ] Bag for disposal</td>
<td>[ ] No Pesticides in animal area</td>
</tr>
<tr>
<td>[ ] Necropsy</td>
<td>[ x ] Necropsy</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Infectious Agents?</th>
<th>[ ] Yes</th>
<th>[ x ] No</th>
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<tbody>
<tr>
<td>Radioisotopes?</td>
<td>[ ] Yes</td>
<td>[ x ] No</td>
</tr>
<tr>
<td>Chemical Carcinogens?</td>
<td>[ ] Yes</td>
<td>[ x ] No</td>
</tr>
<tr>
<td>Toxic Chemicals?</td>
<td>[ ] Yes</td>
<td>[ x ] No</td>
</tr>
</tbody>
</table>

Agent(s):
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 ability of sperm to penetrate and traverse cervical mucus is a crucial aspect of fertilization in primates. Many factors can affect sperm progression through the cervix; including anti-sperm antibodies, changes in visco-elastic properties of mucus, and capacitation state or surface properties of sperm. Understanding these factors and how to manipulate them presents opportunities to develop new contraceptive methods. We are presently looking at proteins of epididymal origin that coat the surface of sperm which appear to change in their properties during capacitation. Preliminary evidence suggests that pharmacological modification of these proteins, inducing a “pre-mature” capacitation, may inhibit mucus penetration by sperm. Furthermore, these surface proteins may also serve as a potential target for a “mucin-based” contraceptive vaccine.

The objective of this study is to determine if specific sperm coating proteins are removed or modified during sperm transit through the cervix. Findings should prove useful in understanding the role of these proteins in regulation of capacitation as influenced by cervical mucus in vivo as well as indicating the appropriateness of these proteins as contraceptive targets.

b) Procedures employed in this project:

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

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

** 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.)

2 hours post-coitus (females are mated according to standard procedures used for the CRPRC breeding colony), females are briefly anesthetized with ketamine hydrochloride (10 mg/kg body weight) after which, a pediatric proctoscope is inserted into the vagina. Sperm are collected from the vaginal vault by flushing with 2-3 ml of sterile saline using a blunt ended plastic disposable pipet. Immediately afterwards, females are returned to their cages. At 18-24 hours post-coitus, females are anesthetized with ketamine as before for collection of cervical mucus. Following insertion of the pediatric proctoscope into the vagina, a 10 cm long polyethylene catheter (I.D.=1.19mm; O.D.=1.70mm) is guided to the cervical region. A stainless steel stylet fed into the catheter facilitates the insertion of the last 1 cm section of the catheter into the cervical os. Once the catheter is in position, the stylet is removed and gentle suction is applied with a 10 cc syringe attached to the end of the catheter. Typically, 5-8 cm column of cervical mucus is recovered per female. While still anesthetized, females are prepared for ultrasound guided aspiration of uterine contents. The procedure is performed aseptically using a real-time mechanical sector scanner. Monkeys will be placed in the supine position and the “free-hand” method is used for uterine punctures. The ultrasound-directed transabdominal uterine puncture is made using a 25 gauge x 3” needle attached to a 1cc syringe and fluid is aspirated with gentle suction. Typically, 20-50ul of fluid is collected.

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.

<table>
<thead>
<tr>
<th>Group</th>
<th>Procedures / Drugs</th>
<th>Number of Animals</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vaginal Lavage</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>Cervical Mucus Collection</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>US-guided Uterine Fluid Collection</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>

Categories of invasiveness

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Little or no discomfort or stress&lt;br&gt;&lt;br&gt;<strong>Examples:</strong> 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.</td>
</tr>
<tr>
<td>2</td>
<td>Minor stress or pain of short duration&lt;br&gt;&lt;br&gt;<strong>Examples:</strong> 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</td>
</tr>
<tr>
<td>3</td>
<td>Moderate to severe distress&lt;br&gt;&lt;br&gt;<strong>Examples:</strong> 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</td>
</tr>
<tr>
<td>4</td>
<td>Severe pain near, at or above the pain tolerance threshold&lt;br&gt;&lt;br&gt;<strong>Examples:</strong> 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.</td>
</tr>
</tbody>
</table>

Further descriptions of these categories are included in the instructions following this document.
Cynomolgus monkeys are most appropriate for this study and future contraception studies because they are reproductively similar to humans for which contraceptive methodologies are ultimately being developed. For this pilot study, observations from 2 to 3 peri-ovulatory females should be sufficient to confirm the presence of sperm coating proteins following transit through the cervix. From previous studies, fewer than 1/2 of all mating attempts result in fertilization. This is due in part to the fact that cycle day estimates are not always an accurate determination of timing of ovulation. We would therefore like to collect samples from 6 females in order to increase the likelihood that at least 2-3 of those will be periovulatory, and therefore present conditions optimal for sperm transport through the cervix.

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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Route</th>
<th>When and how often will it be given?</th>
</tr>
</thead>
<tbody>
<tr>
<td>M fascicularis</td>
<td>Ketamine</td>
<td>10</td>
<td>IM</td>
<td>Twice; once at time of vaginal lavage, and once at time of mucus/uterine fluid collection</td>
</tr>
</tbody>
</table>

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 adverse effects from vaginal lavage, cervical mucus collection, or ultrasound guided aspiration of uterine fluid. We have collected vaginal fluid and mucus from dozens of females in past protocols (AC&U# 8159, 8854, 9051, and 9464) with out any signs of negative side effects. Uterine aspirations were conducted in a 10 females at CRPNC with no negative side effects (et al., 1989).

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.

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  [x] 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.

Death is not an endpoint in this study. Euthanasia by clinician’s advice.

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 (email: jawelsh@ucdavis.edu) or http://www.vetmed.ucdavis.edu/Animal_Alternatives/main.htm (email: mwwood@ucdavis.edu)

What was the date on which you conducted this search?  4/30/03

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

<table>
<thead>
<tr>
<th>Database Name</th>
<th>Years Covered</th>
<th>Keywords / Search Strategy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current Contents</td>
<td>1990-2003</td>
<td>monkey, cervical mucus, uterus, collection</td>
</tr>
<tr>
<td>PubMed</td>
<td>1990-2003</td>
<td>Primate, cervical mucus, uterus, collection</td>
</tr>
</tbody>
</table>

What were your findings with respect to alternative methodologies?

The collection techniques is based on methods developed at CRPRC. (et al., 1989; and et al., 1989). There are no other published methods for either cervical mucus or uterine fluid collection in primates that are less invasive.

Has this study been previously conducted?  [ ] Yes  [x] 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?

Such circumstances are not expected during this study. In the unlikely event that complications arise, euthanasia would be performed on clinician’s advice.

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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Method</th>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>route</th>
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</thead>
<tbody>
<tr>
<td>Cynomolgus</td>
<td>As per CRPRC guidelines</td>
<td>Pentobarbitol</td>
<td>60 mg/kg</td>
<td>IV</td>
</tr>
</tbody>
</table>

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

Animals will be returned to colony.
**Project Roster:** Please provide the names of all the individuals who will work with animals on this project. This page will not be made available to the public. Give either the University Employee ID # or a valid UC Davis email address so that we can document training and occupational health compliance for regulatory agencies. Include all investigators, student employees, post-doctoral researchers, staff research associates, post-graduate researchers and laboratory assistants who will actually work with the animals. You don't need to include the staff of the vivarium in which your animals will be housed.

The principal investigator is responsible for keeping this roster current. If any staff is added or subtracted from this project, you must amend the protocol by sending the campus veterinarian a memo describing any changes.

<table>
<thead>
<tr>
<th>Last Name</th>
<th>First Name</th>
<th>Middle Name</th>
<th>UC ID Number or SSN</th>
<th>Email Address</th>
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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.

---

Principal Investigator | Rank / Title | Date
---|---|---

Committee Use Only Below

** Conditions necessary for Committee Approval:

---

Final Disposition of this protocol:

_________ Approved

_________ Not Approved

_________ 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.

---

Campus Veterinarian | Date
---|---
Hi,

I have revised the protocol (attached) according to comments 1-3 below. Thanks for the update, we will be sure to allow for the longer processing time on future protocol approvals.

At 07:35 AM 5/27/2003 -0700, you wrote:

Hi,

I sent the questions out late last week. We have been experiencing an increase in protocol submissions, so I advised to inform the primate users that one should expect a longer turn around time now. I used to be able to process most protocols in less than two weeks, but all of the staff in our office are buried in protocols, so the real time for processing now holds true. You should expect 4-6 weeks for processing now (actually that should be the norm and if sooner, all the better).

If you can have the revised protocol to me on or before noon today, I can add the protocol to next weeks committee agenda. Otherwise it will be considered on the agenda of 6/19.

Regards

Please find attached a copy of the questions I sent out last week.

Hi,

I have received and pre reviewed the recently submitted protocol which has been assigned accession number 10629 for future reference. I have attached a copy of the protocol with the number embedded for ease of making revisions.

For this protocol to be considered on the June 5th committee agenda, please forward the revised protocol to me on or before noon, May 27th.

Thanks in advance,

Protocol 10629 ( )

1. There were a few boxes left blank. Please complete the following sections: housing location - both overnight as well as day; Who will maintain the animals?

2. In section c, you mention that the animals will receive ketamine prior to their procedures. Will the animals be fasted prior to the ketamine anesthesia? Please clarify.

3. In section i, you have included a part of a paragraph that does not relate to this protocol. The first sentence is the only one that applies to the animals on this study. Since this section asks for the adverse effects associated with the study as it relates to the animals and not a justification, please delete the last two sentences.