PROTOCOL FOR ANIMAL USE AND CARE

CNPRC

Investigator

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

Species (common names): Rhesus Macaque  Number: 30  Source: CRPRC

Project Title: Macaque Sperm Cryopreservation

Overnight housing location: CRPRC  Day use: CRPRC

Animals will be maintained by: [ X ] 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.

Twenty reproductively sound female macaques will be superovulated using a combination of recombinant human gonadotropin hormones. Injections will be intramuscular once or twice daily for 6-9 days. Urine will be collected daily for determination of estrogen levels to assess ovarian stimulation. Ten male macaques will be electroejaculated using routine procedures.

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)

Sick Animals  Dead Animals  Pest Control

[ X ] Call Investigator  [ X ] Call Investigator  [ X ] Call Investigator
[ X ] Clinician to treat  [ ] Bag for disposal  [ X ] OK to use pesticides
[ ] Terminate  [ ] Save for Investigator  [ ] No Pesticides in animal area
[ ] Necropsy  [ X ] Necropsy

Hazardous Materials (only if in the animal room):

Infectious Agents?  [ ] Yes  [ X ] No
Radioisotopes?  [ ] Yes  [ X ] No
Chemical Carcinogens?  [ ] Yes  [ X ] No
Toxic Chemicals?  [ ] Yes  [ X ] No
Funding source: NIH-NCRR
Previously approved? [X] Yes [ ] No

Is the project already funded? [X] Yes [ ] No
Previous protocol number (if any):

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

[ ] Lab Animal Health Clinic (2-0514)
[ ] VMTH Large Animal Field Service (2-0292)
[ ] California Primate Research Center (2-0447)
[ ] Another Veterinarian

If you checked “Another Veterinarian”, please provide:

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

If your veterinarian is not affiliated with one of the three service units listed above, please contact the campus veterinarian, 2-2357 (email pctillman@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.

Our long range goal is to develop simple procedures for gamete preservation and long-term storage that can be applied to sperm of all non-human primates. The objective of this particular application is to optimize protocols for cryopreservation and/or freeze-drying and ICSI (intracytoplasmic sperm injection) of macaque sperm, and to demonstrate their efficiency in terms of the numbers of embryos produced in vitro. The central hypothesis for the proposed research is that the genetic material of macaque sperm can be preserved by simple methods which do not require that sperm viability and motility be maintained. The rationale for focusing this application on the combined approaches of simple sperm preservation techniques and ICSI is that such methods are likely to be applicable to sperm of all non-human primates with little or no modification, and that they can be easily learned and applied in other centers. Consequently, we expect that resulting protocols will allow for long-term preservation of primate stock as valuable models of human disease.

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

** 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.)

Female macaques will be superovulated using injections of recombinant human gonadotropins as well as concurrent treatment with Antide®, a GnRH antagonist. All animals will have overnight urine collected beginning two weeks following the onset of menses. On the morning of day 1-4 following the onset of the next menses, females will be restrained, a 2-3 ml blood sample will be obtained, and the first FSH injection (30 IU, im) will be administered while cage-restrained. Urine will be continued to be collected daily throughout the study from the cage pan each morning without animal restraint. Females will receive FSH injections twice daily at 0800h and 1600h and Antide, once daily (0.5mg/kg, im), through treatment days 1-6 and FSH/LH (30 IU each) through treatment days 7-9. All injections will be administered in less than 0.4ml total volume. Animals will be ultrasounded for follicular development on treatment day 7. If follicular size is less than 4mm, 2-3 additional days of FSH injections will be administered. Those animals will be re-ultrasounded and if poor follicular development is documented, they will be dropped from the study for this menstrual cycle. For those animals continuing, on treatment day 11, they will be anesthetized using ketamine and placed under dorsal recumbency with isoflurane inhalation anesthesia for laparoscopic oocyte retrieval. A 1 cm midventral incision will be made into the skin and fascia and a Verres needle will be introduced into the abdominal cavity. Carbon dioxide will be used to insufflate the abdomen to approximately 12mmHg pressure and the Verres needle will be removed. A 5mm trocar will be introduced into the same incision and the 5mm laparoscope will be introduced. A 1 cm incision will be placed into the right caudal abdomen and an addition 5mm port will be introduced for grasping forceps. Each ovary will be suspended sequentially for oocyte retrieval by atraumatic forceps at the ovarian proper ligament. A 3 inch 22 gauge needle attached to mild vacuum pressure will be introduced into the abdomen and each visible follicle will be aspirated into a 15 ml sterile tissue culture tube containing 5 ml TALP medium. The instruments will be withdrawn and each incision will be closed using standard procedures. The females will then be recovered and given oxymorphone (0.15 mg/kg, im) for postoperative pain as needed for 1-2 days. Each female will be used for a maximum of three cycles of superovulation and laparoscopy per year. Concurrently with oocyte retrieval, male monkeys will be subjected to routine semen collection using chair-restraint and electroejaculation. Each male will not have semen collection more than three times weekly. Semen will be transported to our laboratory and will be used for oocyte injections.

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>Superovulation/laparoscopic oocyte retrieval</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Electroejaculation</td>
<td>10</td>
<td>1</td>
</tr>
</tbody>
</table>
Categories of invasiveness

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
</table>
| 1        | Little or no discomfort or stress  
  **Examples:** 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  
  **Examples:** 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  
  **Examples:** 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  
  **Examples:** 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 macaque is the most appropriate animal model for this study because of its similarities in reproduction to the human. Such similarities include ovarian function, spontaneous ovulation, the presence of a reproductive cycle with similar hormonal dynamics and specifically a drop in fecundity with increase in age. In vitro fertilization and ICSI protocols are also similar between human and monkey models.

We plan to stimulate 20 females per year using gonadotropin treatment in order to have successful ovarian superovulations in at least 10 animals. This will provide us with approximately 10 oocytes per cycle and with 3 stimulation cycles per year, this is likely to result in 30 oocytes per female per year, or 300 oocytes. The latter number of oocytes is required for ICSI success rates of at least 50% using the 10 males already in use as semen donors at CRPRC. Ten males will be required for this study since ejaculates from 5-10 ejaculates per week will be required for sperm studies prior to and including the ICSI studies. Males will be used a maximum number of 3 times per week and only on alternate days. Consequently, male overuse will be avoided at all times during the study.

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

<table>
<thead>
<tr>
<th>Building:</th>
<th>CRPRC</th>
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<tbody>
<tr>
<td>Room:</td>
<td>Surgical Suite</td>
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</table>

Who will be the surgeon? Dr. and CRPRC veterinary staff

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. mulatta</td>
<td>ketamine</td>
<td>10</td>
<td>IM</td>
<td>1 time/laparoscopy</td>
</tr>
<tr>
<td>M. mulatta</td>
<td>atropine</td>
<td>0.04</td>
<td>IM</td>
<td>1 time/laparoscopy</td>
</tr>
</tbody>
</table>
M. mulatta  isoflurane  To effect  inhal  1 time/laparoscopy
M. mulatta  oxymorphone  0.15  IM  3x/day, 2-3 days post-laparoscopy for pain

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?

Not applicable, no neuromuscular blocking agents will be used

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

Pulse-oximetry, standard anesthesia monitoring

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)

Daily monitoring of all animals on this study will ensure maintenance of health and animal well-being. Animals may experience some post-laparoscopy discomfort. We do not anticipate any adverse effects associated with urine collections and ultrasound exams.

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 post-surgical discomfort will be alleviated with oxymorphone.

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.

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? 12/18/02

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>
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<tbody>
<tr>
<td>Medline</td>
<td>1995-2002</td>
<td>Primate, cryopreservation, sperm, gamete</td>
</tr>
<tr>
<td>Web of Science</td>
<td>1995-2002</td>
<td>Primate, cryopreservation, sperm, gamete</td>
</tr>
</tbody>
</table>
What were your findings with respect to alternative methodologies?

No alternative methods were found by which to conduct the present study.

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?

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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Method</th>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>route</th>
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<tr>
<td>M. mulatta</td>
<td>As per CRPRC guidelines</td>
<td>Pentobarbitol</td>
<td>60 mg/kg</td>
<td>IV</td>
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m) Surplus animals: What will you do with any animals not euthanized at the conclusion of the project?

All animals will be returned to the CRPRC colony.
n) **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>
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<tr>
<th>Last Name</th>
<th>First Name</th>
<th>Middle Name</th>
<th>UC ID Number or SSN</th>
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**Occupational Health Program:**

Supervisors must enroll their employees in the campus Occupational Health Program if the workers are at increased risk of illness or injury (such as allergy, physical injury, or infectious disease) because of their work. Enroll workers by having them complete an "Animal Contact History Form", available from Employee Health Services (phone 752-2330). For further information, visit our web site at [http://ehs.ucdavis.edu/animal/health/](http://ehs.ucdavis.edu/animal/health/) or read the UC Davis Policy & Procedure Manual 290-25.

**Training:**

Supervisors are responsible for insuring that their employees are adequate trained, both in the specifics of their job and in the requirements of the Federal Animal Welfare Act. EH&S offers free, basic wet labs in laboratory animal handling and techniques, and lecture format classes in the requirements of the Animal Welfare Act. To schedule a class for your unit, contact EH&S at 2-2364. Information is available on the world wide web at [http://ehs.ucdavis.edu/](http://ehs.ucdavis.edu/).
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