### Investigator Contact

| Last Name: | Last Name: |
| First: | First: |
| Middle: | Middle: |
| Email: | Email: |
| Department: | Department: |
| Phone / Fax: | After hrs. #: |

### Species (common names)

<table>
<thead>
<tr>
<th>Species</th>
<th>Number</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhesus females-oocytes</td>
<td>53 per year</td>
<td>CNPRC</td>
</tr>
<tr>
<td>Rhesus females-embryo transfer</td>
<td>30+10 offspring = 40 per year</td>
<td>CNPRC</td>
</tr>
<tr>
<td>Rhesus males - semen</td>
<td>6 per year</td>
<td>CNPRC</td>
</tr>
</tbody>
</table>

### Project Title

Macaque follicle aspiration and embryo transfer

### Overnight housing location:

<table>
<thead>
<tr>
<th>CNPRC</th>
<th>Day use:</th>
</tr>
</thead>
</table>

### Animals will be maintained by:

- [x] Vivarium
- [ ] Investigator

### 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.

Adult female monkeys are injected with gonadotropin hormones twice daily for 7 days to allow the development of multiple ovarian follicles. The monkeys are anesthetized and the contents of the follicles are aspirated by ultrasound guidance. Some of the embryos that result from the in vitro fertilization procedures will be transferred under ultrasound guidance to unrelated recipient females.

### 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.

None

### Other instructions for animal care staff:

(check applicable entries)

- [x] Call Investigator
- [x] Call Investigator
- [ ] Call Investigator
- [ ] Save for Investigator
- [ ] Bag for disposal
- [ ] 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

Agent(s):
Funding source: NIH
Previously approved? [x] Yes [ ] No

Is the project already funded? [x] Yes [ ] No
Previous protocol number (if any): 9207

What Veterinarian or veterinary clinic will provide care for your animals? (check one)
[ ] Lab Animal Health Clinic (2-0514)  [ ] California Primate Research Center (2-0447)
[ ] VMTH Large Animal Field Service (2-0292)  [ ] 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 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.

Oocytes will be obtained from rhesus females to develop protocols for macaque oocyte freezing and in vitro maturation (IVM) and to produce embryos to study early embryonic development and gene expression. These studies are important to improve the macaque research model and can be used to preserve endangered primate species. Rhesus macaques are the preferred model for studies that are investigating human reproduction because of the many similarities among primate species.

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.
[ ] Survival surgical procedures
[ ] Death as an endpoint (see i below)

[ x ] catheters, blood collection, intubation
[ ] Multiple survival surgery
[ ] Trapping, banding or marking wild animals

[ ] Prolonged restraint. (8 hrs+)
[ ] Behavioral modification.
[ ]

[ x ] 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.)

Rhesus Females – Oocyte donors (N=53):

Control – in vivo maturation of oocytes (N=25)
Macaque FSH, CG – testing new hormone source (N=3)
IVM – in vitro maturation of oocytes (N=25)

Superovulation and follicle aspiration: Adult female rhesus macaques (total of 53) from the two groups above are given twice daily intramuscular (IM) injections of recombinant human follicle stimulating hormone (rhFSH, 37.5 IU/day, Organon) for seven days. If available (see below), recombinant macaque FSH will be given rather than human FSH. Antibodies (Ares-Serono, 0.5 ng/kg body weight subcutaneous) is given on the days when the females are receiving FSH injections to prevent spontaneous ovulation. On day 7, for control experiments in which oocytes are matured in vivo (“Control”), monkeys (N=25) are given an IM injection...
of recombinant human chorionic gonadotropin (rec hCG; 1,000 IU; Serono) to simulate the natural mid-cycle luteinizing hormone surge and to promote the final maturation of the follicle. Monkeys for the IVM experiments (N=25) do not receive rec hCG. The monkeys are fasted the night before the aspiration procedure and anesthetized with ketamine or telazol the following morning. After delivery to ultrasound, the monkeys are aseptically prepared for the sterile transabdominal aspiration procedure using established, published ultrasound-guided techniques. Using a freehand approach, follicles are aspirated under direct ultrasound imaging by guiding the aspiration needle from follicle to follicle while aspirating the contents with mild negative pressure, comparable to the procedure used in humans. At the end of the procedure, the monkeys are returned to their home cage and observed periodically until awake. The procedure takes approximately 15-20 minutes from delivery of the animal to the ultrasound suite to return of the animal to the home cage. To date, we have observed no adverse effects of this procedure on the monkeys (in 11 years we have performed more than 300 aspirations). It has been shown in our previous studies that the monkeys will develop antibodies to the human hormones and cannot be hormonally stimulated more than 5 or 6 times (average is about 3 times). All animals are given one full menstrual cycle with no interventions before another stimulation cycle is attempted. We have shown in cycles following the menstrual cycle in which this procedure is performed, monkeys have conceived and the pregnancy has proceeded uneventfully to term with normal infants, further indicating no adverse.

Macaque gonadotropins for superovulation. As noted above, because the only hormones currently available are human hormones, not macaque, the monkeys will eventually develop antibodies to the human hormones after 3 to 5 cycles, and generally will become unresponsive to the hormones. We have a cell line producing both macaque FSH and chorionic gonadotropin (CG) with the goal of developing techniques for use of macaque hormones and avoiding the generation of antibodies to the human hormones. Current procedures are focused on obtaining purified protein for this purpose. Once available, macaque FSH and CG will be administered to 3 female rhesus macaques at the same schedule and equivalent dose as currently used with human FSH and CG (7.5 µg in 0.2 cc/animal, twice daily, IM injection). Animals will be monitored by ultrasound (under ketamine or telazol anesthesia (following overnight fast, food offered upon awakening), beginning on Day 4 of treatment. Each animal will be examined by ultrasound 3 or 4 times over a 5 day period: 1 or 2 observations to ensure there is follicular growth after day 4, then 2 exams in a row prior to aspiration to assure follicles have reached the expected size. In prior years, animals were monitored in this manner during administration of the human hormones, however, with experience, it became unnecessary to monitor with this frequency. Since we are assessing new hormonal agents that are monkey-specific, it is necessary to return to this established protocol. If ovarian follicular development is similar to that obtained with human hormones, ultrasound-guided aspiration will proceed as specified in the existing protocol. If follicular development is slower than expected, additional days of FSH treatment will be added to the superovulation protocol, for a maximum of 10 days of FSH. If no follicular development is detected by Day 4, the dose of FSH will be increased (doubled). If follicular development is greater or faster than expected, the dose of FSH will be reduced by half. Animals requiring 10 days of FSH will have a maximum of 5 ultrasound exams (beginning on Day 4) over a 7 day period. Because the exams will take place in one week of the menstrual cycle and animals will have a cycle (month) off before being treated again, it is unlikely that any adverse affects on long-term nutrition will occur. If treatment is successful in stimulating the development of multiple follicles in the initial 3 females designated for this purpose, the use of macaque hormones will replace human hormones for ALL animals assigned to these studies. However, it will continue to be important to monitor the serum antibody titers of more than the 3 females first assigned to receive macaque hormones so that we can get more accurate data through multiple cycles to determine if antibodies to the macaque hormones are being formed. Therefore, blood (~3 cc by arm pull when animals are in the cage using the squeeze mechanism at beginning of the
cycle, and ~3 cc from a femoral vein at end of FSH, CG treatment while under ketamine or telazol for ultrasound guided aspiration of follicles) will be collected at the beginning and end of each treatment cycle to monitor the formation of antibodies in at least 10 animals if we beginning using macaque hormones to stimulate all animals. If the macaque hormones are successful in stimulated multiple cycles without antibody formation, we should be able to use all of the female rhesus designated as oocyte donors for multiple cycles of oocyte aspiration.

In our previous studies, a small number of animals were stimulated many times with human hormones and had normal pregnancies following 8 to 10 stimulation and aspiration cycles. Therefore, 10 cycles of stimulation and oocyte aspiration will be the maximum for each animal. We will continue to monitor the pregnancy rate of animals after they are released from this project to be sure that fertility is unaffected.

Rhesus Males - Semen Collection (N=6): The males maintained for semen collection wear a light-weight metal alloy collar to facilitate moving the monkey to the primate chair. Semen will be collected, using a published protocol, from previously chair-trained adult male rhesus macaques by penile cuff electroejaculation a maximum of 3 times per week. This project will use up to 6 male rhesus per year. We anticipate that once males are assigned, they will be used as semen donors for many years, therefore it is unlikely that 6 different males will be used each year.

Rhesus Females - Embryo Transfer (N=30+10 offspring=40): This procedure will also require monitoring urinary hormone levels and one blood sample in addition to the embryo transfer procedure. We estimate that approximately 30 embryo transfers will be performed each year to obtain approximately 10 pregnancies. Adult macaque females that have not been mated at mid-cycle will be monitored for urinary hormones for one week by collection of urine from the cage pan under the cages. Embryo transfer will be performed 5 days following the mid-cycle estrogen surge. Any animals that do not become pregnant will be returned to the colony as soon as menses occurs. Females that become pregnant will be monitored periodically (every 2 to 4 weeks under ketamine) by ultrasound to monitor development of the fetus. Once delivery occurs and the newborn is assessed by visual examination, they will be released to the colony.

1. Blood sampling. All embryo transfer recipients (N=30) will have one blood sample (5 cc, femoral vein) drawn on day 7 after transfer for pregnancy detection by mCG and for DNA typing to prove that any resulting offspring are unrelated to the recipient females. (~3 cc by arm pull when animals are in the cage using the squeeze mechanism).

2. Urine collection. Urine collection from pans placed below each cage will be performed daily for one week during each menstrual cycle for all embryo transfer recipients (N=30). The urine samples will be analyzed to confirm the timing of the menstrual cycle to optimize the time for embryo transfer.

3. Embryo transfer (N=30). Monkey embryos used for this procedure will be the result of in vitro fertilization and in vitro embryo culture. The embryos will be loaded into fine polyethylene tubing. We will use a sterile, non-surgical, ultrasound-guided procedure for embryo transfer that is similar to the published procedure used for intrauterine inseminations where the animal is anesthetized with ketamine (after an overnight fast), aseptically prepared, and the tubing containing the embryo is threaded through a needle placed transabdominally under ultrasound guidance with the tip into the uterine cavity at the level of the fundus. The embryo is gently injected into the cavity, the catheter removed, then the uterus is monitored post-transfer to assess the uterine cavity and confirm the site of injection. At the end of the procedure, the monkey is returned to the home cage and observed periodically until awake. The procedure takes ~15 to 20 minutes from delivery to the ultrasound suite to return to the home cage. To date, only a few procedures have been performed and no pregnancies have been obtained because low quality embryos were used.

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>Oocyte donors -Controls – rec FSH, Antide, and CG</td>
<td>25</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Oocyte donor - IVM - rec FSH, Antide</td>
<td>25</td>
<td>2</td>
</tr>
</tbody>
</table>
Macaque FSH, CG – testing new hormone source  

Embryo transfer recipients (30) + 10 offspring  

Males for semen collection – no treatment  

<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 group was the minimum number necessary to achieve sound scientific results?

**Choice of species:** The purpose of this study is to develop methods for maturation of cryopreservation of macaque oocytes to improve the macaque model for research. The best species to use for this study, therefore, is macaques. Additionally, this research will provide information that can be applied to humans. Human and non-human primates (such as rhesus monkeys), share many similarities of oocyte and embryo biology that other species do not share. Some examples are: 1) usually, only 1 oocyte is matured and ovulated; 2) the oocytes are very susceptible to temperature changes; 3) the embryonic genome expression begins around the 8-cell stage of embryo development; and 4) the embryo secretes a hormone (chorionic gonadotropin) as a signal for maternal recognition of pregnancy. This unique combination of these characteristics (and others not mentioned) make the rhesus monkey an especially good model for human reproduction.

**Superovulation and follicle aspiration:** To develop methods for IVM, oocytes will be collected from females that have had only part of the usual hormone doses for producing in vivo matured oocytes. Initially, only the rec hCG dose will be eliminated, then, once we have developed culture media that can support the final phase of oocyte maturation, the number of days of rec hFSH injections will also be reduced. However, these experiments require an equal number of control (in vivo matured) oocytes for comparison. The experiments on cryopreservation of oocytes will require both in vivo matured and immature oocytes. The experiments will require a total of 1200 control and 1200 IVM oocytes per year. Our previous experience is that we obtain about 20 usable oocytes per cycle. Although we can use each female more than once, we now know from experience that we average approximately 3 or 4 cycles per female. Therefore, we will require 21 females for each treatment group (20 eggs x 3 cycles x 21 females = 1260 oocytes). Also, we know that each
year we have a few females that do not respond to the hormone injections and produce so few oocytes that they are not useful for repeated experiments. Unfortunately, there is no way to predict this poor response, so we have increased our yearly requirement to 25 for each treatment to allow for 4 poor responders per year. Even if we switch to macaque hormones for all stimulation cycles, it is likely that we will still have poor responders, just as women can respond poorly when using homologous (human) hormones.

**Semen Collection:** It is necessary to utilize rhesus macaques for this protocol because the semen collected is used for in vitro fertilization of rhesus oocytes. Using a maximum of six animals per year insures that an adequate number of animals are available so that no one monkey is used more than 2 times per week.

**Embryo Transfer:** The purpose of this project is to develop reliable protocols for in vitro maturation and cryopreservation of macaque oocytes. In order to fully evaluate the viability of embryos that result from these oocytes, we must transfer some embryos to recipient females to determine if the oocytes are capable of complete development, i.e. they can result in healthy offspring. These studies should require 10 or less offspring per year. However, because embryo transfer in primates is relatively inefficient, less than 30% success rate, we must plan on performing approximately 3 times the number of transfers compared to the offspring needed. Therefore, producing 10 offspring per year will require approximately 30 transfers. It is possible than we may need fewer offspring in some years or that the success rate will be improved as we progress in our studies, therefore it is likely that fewer than 30 procedures will actually be performed.

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

<table>
<thead>
<tr>
<th>Building</th>
<th>Room</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>

Who will be the surgeon?

<box>g) **Anesthetics, Analgesics, Tranquilizers, Neuromuscular blocking agents:**</box>

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>rhesus</td>
<td>Ketamine hydrochloride</td>
<td>10</td>
<td>IM</td>
<td>Once per menstrual cycle.</td>
</tr>
<tr>
<td>Rhesus</td>
<td>Telazol</td>
<td>5–8</td>
<td>IM</td>
<td>Once per menstrual cycle.</td>
</tr>
</tbody>
</table>

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)
Superovulation and follicle aspiration: Because follicle aspiration is an ultrasound-guided procedure, it is relatively non-invasive, and because we plan to rest each animal for one menstrual cycle between treatment cycles, there should be no complications as a result. There have been clinical reports of women undergoing up to 10 cycles of superovulation and ultrasound-guided follicle aspiration without ill effects. We now have 5 years of experience with animals receiving repeated stimulation protocols and have seen no adverse effects. We have monitored the animals that have had multiple follicular aspirations and they are able to have normal term pregnancies following the procedures. This protocol will involve up to 5 ultrasound exams, and consequently, ketamine anesthesia, in a one-week period during every other menstrual cycle. In the past, this number of times for ketamine anesthesia has not been a problem for poor appetite or long-term nutritional effects. However, if poor appetite is reported for any animal for more than 3 days, supplements will be given as long as recommended by the veterinary staff.

Semen Collection: This method has been used routinely at CNPRC for many years with good results. The males are not anesthetized during semen collection, but it has been reported that human volunteers did not find the penile cuff method of electroejaculation to be painful. Additionally, after the macaques are trained to the procedure, they cooperate fully during the transfer from cage to chair restraint. We have improved the procedure by using EEG gel material, instead of metal, for the electrode material. The gel-electrode material has eliminated the risk of tissue injury of the penis and we have had no lesions in over 12 years that semen has been collected by this method.

Embryo Transfer: Because embryo transfer is an ultrasound-guided procedure, it is relatively non-invasive. Dr. has over 18 years of experience in injecting into and aspirating from the uterine cavity by ultrasound guidance and thus, no adverse effects are expected. Each transfer recipient will be monitored for a minimum of two weeks and if non-pregnant will be immediately released to the colony. Although not likely based on extensive experience, since the procedure is performed under continuous ultrasound guidance, this monitoring provides the opportunity to determine any potential adverse events, should they occur.

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.

Although we do not anticipate any adverse effects based on our previous experience, analgesics would be administered to alleviate any potential pain or discomfort. Analgesics will be administered at the discretion of the CNPRC veterinary staff in consultation with the investigator.

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? May 2003

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>Medline</td>
<td>1980-03</td>
<td>Maturation, IVM, embryo culture, cryopreservation, freezing, culture media, embryo development, amino acids, energy substrates, oocytes, embryos,</td>
</tr>
<tr>
<td>Biosis Previews</td>
<td>? to present</td>
<td>Same as above</td>
</tr>
<tr>
<td>CRISP at NIH</td>
<td>current</td>
<td>same as above to determine if any similar grants had been recently funded whose work might not yet have appeared in the literature.</td>
</tr>
<tr>
<td>Current Contents</td>
<td>current</td>
<td>Same keywords, but also specific journals</td>
</tr>
</tbody>
</table>

What were your findings with respect to alternative methodologies?

This grant was awarded to specifically develop methods for the in vitro maturation and cryopreservation of macaque oocytes. No alternative method or model would allow us to develop these methods.

Every few months the literature is searched and reviewed in an effort to stay current with the literature. The last such review was May 2003. The NIH grant that funds this work was renewed in 2002, when an extensive literature search was performed.

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?

No animals will be euthanized. All animals are returned to the colony.

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>
</tr>
</thead>
<tbody>
<tr>
<td>Rhesus</td>
<td>overdose</td>
<td>sodium pentobarbital</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 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</th>
<th>Email Address</th>
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</thead>
<tbody>
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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.

Adjunct Professor  7/1/03

Principal Investigator  Rank / Title  Date

CNPRC Director  Date

Committee Use Only Below

** Conditions necessary for Committee Approval:

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<th>Condition</th>
<th>Approval Status</th>
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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
Pre review questions Protocol 10786

Hi,

I have received and pre reviewed the recently submitted protocol which has been assigned accession number 10786 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 10786 ( )

1. On page 1 the following boxes were left blank. Please complete the overnight housing, day use and animal maintenance boxes.

2. On page 1, the list of animal numbers is a total of 99 primates. Is that number a per year or for the life of the protocol, so 99 total? Please clarify.

3. In section c, there is extensive use of acronyms without definitions. Please define the acronyms at least once for the committee members. What does CG, LH, etc refer to?

4. In the second paragraph of section c, you state that the animals will be monitored by ultrasound daily. Will they be fasted prior to the administration of ketamine or telazol? Please detail everything that happens to the animals.

5. If the animals are to be followed by ultrasound beginning on Day 4, how long will you monitor them? Will they receive anesthesia daily for this period as well as fasting? Will you monitor weight loss if there is a lengthy period of daily anesthetic delivery?

6. In the last paragraph of section c, you mention embryo transfer, but do not discuss fasting or intubation. What about anesthesia? Please expand this section.

7. In section e, you have gone into great detail to justify the animal use, but have not included a statement about why you have chosen the primate. Please expand. Also, where does the experiments on cryopreservation fit into the rationale?

8. In section i, death is an endpoint, you have included information about your literature search. It is confusing why you placed this information in this box. Please delete or relocate to the box after the literature search.
I have incorporated the answers to the questions into the protocol.

1. On page 3, it states that each animal will have 3 or 4 ultrasound exams per cycle of treatment. I have expanded the section to explain that in the case of animals that require 10 days of FSH instead of 7, animals will have a maximum of 5 exams during a 7 day period. Also, that animals will have a cycle (month) off before being treated again.

2. Because the animals will have the exams in a one week period and then have another menstrual cycle without treatment, it is unlikely that there will be significant effects on long-term appetite or nutritional status. We have address this in the section on adverse effects.

Protocol Questions

Hi,

I have received the following questions from CNPRC veterinarians regarding protocol 10786 ( ). Please return the responses and questions to the responses to me on or before noon, Thursday, Aug 14th so I can have the clarification before the 2:30 committee meeting.

Thanks in advance,