PROTOCOL FOR ANIMAL USE AND CARE
Handwritten forms are not accepted

CRPRC

Investigator

<table>
<thead>
<tr>
<th>Last Name</th>
<th>First</th>
<th>Middle</th>
</tr>
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</table>

Contact

<table>
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<tr>
<th>Last Name</th>
<th>First</th>
<th>Middle</th>
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</table>

EXPIRES: ________

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>24</td>
<td>CRPRC</td>
</tr>
<tr>
<td>Rhesus males – semen</td>
<td>3</td>
<td>CRPRC</td>
</tr>
</tbody>
</table>

Project Title

Zona escape in rhesus embryos

Overnight housing location:

CRPRC

Day use only:

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.

The mechanism for rhesus embryos shedding their protein coating prior to implantation, will be studied both in vitro and in vivo. In year 1, standard in vitro fertilization embryos will be evaluated for zona escape in vitro. In year 2, a catheter will be placed in the uterine lumen by ultrasound guidance so sequential samples of uterine fluid can be obtained. In year 3, ultrasound-guided uterine biopsies will be obtained to look at enzyme expression in the endometrium.

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)

<table>
<thead>
<tr>
<th>Sick Animals</th>
<th>Dead Animals</th>
<th>Pest Control</th>
</tr>
</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>[ ] 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):

Infectious Agents? [ ] Yes [ X ] No  
Agent(s):

Radioisotopes? [ ] Yes [ X ] No  
Agent(s):

Chemical Carcinogens? [ ] Yes [ X ] No  
Agent(s):

Toxic Chemicals? [ ] Yes [ X ] No  
Agent(s):

University of California, Davis
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Summary of Procedures:

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

This study will evaluate the mechanism of zona escape in vitro and in vivo for rhesus embryos. In other species, such as hamster, specific proteins are produced by the uterine endometrium at the time that the early embryo escapes from the zona pellucida. This process is also known as hatching. Shortly afterwards, implantation begins. Potentially, this could be a process that is abnormal in some infertile women, but also, this process could be a potential target for contraception. Therefore, it is necessary to determine if an analogous protein occurs in primates. In year 1, we will expose monkey in vitro fertilization embryos to protein isolated from the hamster to determine if the protein improves zona escape. In year 2, catheters will be inserted into the lumen of the uterus so that uterine fluid can be collected three times daily for five days around the time of hatching and implantation. This fluid will be evaluated for the presence of specific proteins. In year 3, biopsies of the uterine endometrium will be obtained with ultrasound guidance at the time point identified in Year 2 to be closest to the time of maximal levels of the protein in the uterine fluid.

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 **
- [x] catheters, blood collection, intubation
- [ ] Prolonged restraint (8 hrs+)
- [x] Fasting prior to a procedure.
- [ ] Food or water restriction
- [ ] Non-recovery surgical procedures
- [ ] LD 50 or ID50 studies.
- [ ] Survival surgical procedures
- [ ] Multiple survival surgery
- [ ] LD 50 or ID50 studies.
- [ ] Survival surgical procedures
- [ ] Behavioral modification.
- [ ] 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.
Uterine catheter placement (N=8): The procedure will be performed just after menses, early in the follicular phase of the cycle in which uterine fluid will be collected. The animals will be fasted the night before the procedure and anesthetized with ketamine or telazol. The animal will be prepared for a sterile procedure by established techniques. An 18 gauge x 3 inch needle will be placed transabdominally with ultrasound guidance into the lumen of the cervix near the internal os. Small gauge tubing (PE40) will be threaded through the needle and the end will be advanced into the uterine lumen using established techniques. The needle will be removed, leaving the catheter in place. The free end of the tubing will be attached to a sterile “port” that can be repeatedly sampled. The exact volume of the tubing and port will be measured. A small incision will be placed in the abdominal or inguinal region for subcutaneous placement of the port and the incision will be closed with sutures (performed by CRPRC vet staff). Females will be mated with proven male breeders according to standard CRPRC breeding protocols (three times, every other day at mid-cycle). Monkeys will be housed in primate metabolism cages and daily urine samples will be collected to determine the day of ovulation. Beginning on day 5 after ovulation, uterine fluid will be sampled twice daily (7am and 4pm) until day 10 after ovulation. Animals will have uterine fluid sampled without anesthesia while either handheld or in a squeeze cage. A sterile 1ml syringe with a 22g x 1inch needle will be inserted into the subcutaneous port transdermally (no incisions are required to sample the port). After removal of uterine fluid, the tubing will be filled with sterile saline (volume as measured above) to prevent clogging of the tubing due to protein in the uterine fluid. Previous studies of in vivo flushed embryos have determined that zona escape occurs around day 7-8 after ovulation. We have extended the sampling days for two days before and after this window to accommodate potential variation.

Superovulation and follicle aspiration: Adult female rhesus macaques (N=8) will be given twice daily IM injections of recombinant human follicle stimulating hormone (rhFSH, 37.5 IU/day, Organon) for seven days. Antide is given once daily (5 µg/kg, sub cutaneous) to prevent spontaneous ovulatory surges. On day 7, monkeys 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 follicles. The monkeys will be fasted the night before the aspiration procedure and anesthetized with ketamine or telazol the following morning. After delivery to the ultrasound suite, the monkeys are prepared for the sterile aspiration procedure using established ultrasound-guided techniques ( , 1990). Follicles are aspirated aseptically and transabdominally from each ovary with a 20g x 3in needle using established techniques. At the end of the procedure, each monkey will be returned to their home cage and observed periodically until awake. The procedure takes less than 20 minutes from delivery of the animal to the return to the home cage, and 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). In cycles following the menstrual cycle in which this procedure is performed, monkeys have conceived and the pregnancy has gone to term with normal infants, further indicating that no adverse effects are caused by these procedures.

Semen Collection (N=3): The males maintained for semen collection wear a light-weight metal alloy collar to facilitate moving the monkey to the primate chair restraint. Semen will be collected from chair-trained adult male rhesus macaques by penile cuff electroejaculation a maximum of 3 times per week ( et al., 1990). This project will use up to 3 male rhesus per year to assure that some of the embryos are genetically unrelated. We will use each male only a few times for semen collection on this project, so whenever possible, we will use males that are already trained as semen donors at CRPRC.
between individuals and possible sampling errors in determining the day of ovulation. After collections have been completed, the animal will be anesthetized and a small incision will be made in the skin and the tubing and needle hub with silicone gel will be removed.

Uterine biopsies (N=8): Ultrasound guided uterine biopsies will be performed on female rhesus monkeys using established techniques (, unpublished) on the day following ovulation that is determined by the uterine fluid study (above) to be the maximal level of enzyme present in the uterus. Monkeys will be housed in primate metabolism cages and daily urine samples will be collected to determine the day of ovulation. The animals will be fasted the night before the procedure and anesthetized with ketamine or telazol. The animal will be prepared for a sterile procedure by established techniques. The biopsies will be performed transabdominally using an 18 or 20 gauge biopsy needle.

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 for monkey IVF embryos</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Males for semen collection – no treatment</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Uterine fluid collection</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>Uterine needle biopsy</td>
<td>8</td>
<td>2</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?

1. Preimplantation development of primate embryos is a complicated process and many characteristics of their development are unique to human and nonhuman primates. Other model species are not appropriate because of these characteristic differences.

2. The wide range of variation in the biological response of primates requires sufficient numbers in each experimental group.

Superovulation and follicle aspiration: These studies will require embryos from 5 females and also require that embryos develop to the blastocyst stage and subsequently hatch. Half of the embryos will be treated and half controls, so at least 6 blastocysts from each female will be needed. Previous studies at the CRPRC show that 90% of the monkeys respond to gonadotropins and that 100% of these are successfully aspirated for oocyte recovery. The average number of fertilizable oocytes per female is about 15 to 20. Approximately 60% of normal IVF embryos develop to the blastocyst stage and about 80% of those will hatch in vitro. Therefore, we should be able to perform experiments on each of the females, however, because it is possible that females will not respond to the hormones, will not have fertilized oocytes, or will have poor embryonic development, we have planned for 8 females to be superovulated.

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 three animals per year insures that an adequate number of animals are available so that we will have unrelated embryos that exhibit normal levels of variation.

Uterine Fluid Collection: We will need uterine fluid from at least 5 females to determine if the zona escape enzyme is present in rhesus uterine fluid. Because these experiments also depend on animals being successfully bred with preimplantation embryos present, we have planned for 8 females. Because animals will be selected based on previous high fertility and will be monitored for positive breeding, three additional females should be sufficient to assure that 5 animals have embryos present.

Uterine Biopsies: Biopsies from 5 females will be required to evaluate the uterus for the presence of the zona escape enzyme. The uterine fluid studies (above) will confirm that the enzyme is present and the time after ovulation at which the levels of this enzyme are maximal. Because there is the potential for errors in establishing the day of ovulation, we have planned for 8 animals to have this procedure to assure that we have 5 positive samples.
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>rhesus</td>
<td>Ketamine hydrochloride</td>
<td>10 mg/kg</td>
<td>IM</td>
<td>Once</td>
</tr>
<tr>
<td>Rhesus</td>
<td>Telazol</td>
<td>5-8</td>
<td>IM</td>
<td>once</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)

None are anticipated based on our extensive experience.

Superovulation and follicle aspiration: Because follicle aspiration is an ultrasound-guided procedure, it is relatively non-invasive, and because we plan to collect oocytes only once on each animal, there should be few adverse effects. 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 4 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.

Semen Collection: This method has been used routinely at CRPRC 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.

Uterine fluid collection: We do not anticipate any adverse effects. We have previously performed studies that included transient placement of uterine catheters without any evidence of adverse effects. The catheter will actually be placed through the wall of the cervix, and into the lumen of the uterine body, so there is less
chance of uterine reaction. It is likely that the tubing will act much like an IUD birth control device and prevent implantation of the embryo, but have no further effects. Although the placement of the catheter and subsequent fluid collection are performed aseptically, there is a potential for ascending infection. However, the animals will be monitored by ultrasound immediately prior to the beginning of the sampling period. Additionally, they are monitored daily in their home cage by animal care staff. If any signs of infection are seen, antibiotics will be administered.

**Uterine biopsies:** Uterine biopsies have been previously performed with ultrasound guidance without ill effects. This procedure is routinely performed by Dr.  .

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 discomfort. Analgesics will be administered at the discretion of the CRPRC 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:

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

What was the date on which you conducted this search?  

May 2001

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>1980-01</td>
<td>Maturation, IVM, embryo culture, cryopreservation, freezing, culture media, embryo development, amino acids, energy substrates, oocytes, embryos.</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></td>
<td>all, especially pre 1980</td>
<td>found older papers cited in more current articles</td>
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</table>

What were your findings with respect to alternative methodologies?

There are none.
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</td>
<td>60 mg/kg</td>
<td>IV</td>
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<tr>
<td></td>
<td></td>
<td>pentobarbital</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.
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>
<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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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://clueless.ucdavis.edu/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. Autotutorials are also available on the world wide web at http://clueless.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.

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Rank / Title</th>
<th>Date</th>
</tr>
</thead>
</table>

Committee Use Only Below

** Conditions necessary for Committee Approval:

<table>
<thead>
<tr>
<th>Condition 1</th>
<th>Condition 2</th>
<th>Condition 3</th>
</tr>
</thead>
</table>

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.