IACUC Policy 18: Frog Oocyte Harvest

1. Background
Amphibian oocytes are used for studies of molecular biology, embryology and biochemistry. Stage I-VI oocytes are often obtained by surgical laparotomy. Multiple surgeries on a single animal may be justified considering the simplicity of the procedure, the lack of complications when performed by competent personnel, the effectiveness of anesthetic regimens, and reduction in the number of animals needed compared to the number that would be required if only one surgery were permitted.

2. Policy
Five recovery surgeries/animal (with a final 6th terminal surgery) will be permitted.4 Waivers may be granted on an exceptional basis by the IACUC for compelling scientific reasons.

Adequate recovery time shall be allowed between laparotomies. Investigators shall alternate oocyte collection between right and left ovaries and rotate frogs so that the interval between surgeries is maximized. Recovery times shall not be less than one month under any circumstances unless approved by the IACUC.

3. Training
Surgeries must be performed by persons with appropriate training and must use appropriate anesthetic agents, such as tricaine methane-sulfonate (MS-222).

Professional and technical personnel and students who perform anesthesia, surgery, and euthanasia must be trained to accomplish these tasks in a humane and scientifically acceptable manner. The principal investigator must assure the IACUC that the project personnel have demonstrated competency or will be adequately trained to perform anesthesia, surgery, euthanasia and necessary monitoring to the satisfaction of the Attending Veterinarian or a qualified individual designated by the Attending Veterinarian. The LARC veterinary staff is available to provide assistance with, or training in, aseptic and surgical techniques, the proper administration of anesthesia, and euthanasia.

4. Xenopus Oocyte Harvest Recommended Protocol
A. Pre-operative considerations: Frogs are to be fasted for 6-12 hours to prevent emesis during anesthesia. Animals should be individually identifiable (i.e., RFID microchip implanted at the first surgery).

B. Anesthesia and supportive care during surgery: MS-222 can be safely used on Xenopus sp. at a dosage range of between 0.5 and 3 g/L. MS-222 solutions should be buffered to pH 7.0. Dosage selection is dependent upon the weight/size of the frog and the duration of anesthesia required. Once a surgical plane of anesthesia has been reached, (noted by a lack of response to deep pain, i.e. toe pinch) animals are placed in dorsal recumbency on the non-absorbent “blue” side of a clean/unused diaper pad. Optionally, investigators may lay the frog in an ice-filled container covered with plastic wrap (to prevent skin freezing) to induce hypothermia for additional analgesic effects. The frog can be frequently exposed to water containing dissolved MS-222 to maintain current level of anesthesia for long procedures (>30min.) or moistened less frequently for brief procedures (<30min). Frog skin must remain moistened throughout the procedure to prevent desiccation and precipitate complications. Take care not to introduce anesthetic water into the incision as this will prolong recovery from anesthesia.

C. Instrument Sterilization: Surgical instruments must be washed or soaked post-usage to remove all debris. They can be subsequently wrapped or packed appropriately before being sterilized with high heat and pressure or high heat alone (steam autoclave) or adequate exposure to gas (ethylene oxide). Steam is the preferred method (autoclaving). Instruments are to be stored in a dry place in which the integrity of the wrapping or packing material will be maintained for one year (sterilized packages must be clearly labeled with the one-year expiration date). Instruments sterilized in sealed plastic pouches are good for 1 year. Instruments wrapped and sterilized in cloth are good for 30 days. This extends to 6 months if heat sealed in plastic bags, 2 months if tape sealed in plastic bags. Instruments wrapped and sterilized in polypropylene cloth are good for 6 months.
Sterile materials are no longer considered sterile if the external packaging becomes wet or torn. If multiple surgeries are to be performed on different animals, then previously sterilized instruments may be “quick”-disinfected using a glass bead sterilizer (at least 15 sec). Instruments soaked in chemical disinfectants must be rinsed in sterile water or saline solution before their use on animals. No more than five successive surgeries may use instruments “quick”-disinfected as described above.

D. Surgical Skin Preparation: Skin asepsis in Xenopus sp. is not typically required for the most common surgical procedures performed on this species. However, it is recommended that debris should be removed only from the area immediately surrounding the surgical incision site (by a brief rinse with sterile saline, moistened small gauze pad or cotton swab). There is minimal potential for contamination of the surgical site and the development of subsequent post-operative infection if aseptic procedures are employed (use of gloves, a clean lab coat, and sterile instruments, and surgery occurs in a dedicated /previously disinfected surgical area on a clean surface).

If necessary, the recommended product for skin asepsis is 0.75% chlorhexidine or 2mg/L of benzalkonium chloride with a contact time of 10 minutes after removal of excess skin secretion from the surgical area. The surgical site shall be rinsed with sterile saline before the surgical incision is made. Products that contain soaps or detergents must be avoided and iodine-based products must be diluted significantly if they are to be used; e.g., 0.5% betadine solution.

Care must be taken not to scrub the skin too vigorously as this may remove the protective mucous layer, leading to post-operative complications.

E. Surgical Procedure: A small, paramedian, coelomic incision (0.5-2cm) through the skin and muscular layers is made on either the right or left side of the coelom. A portion of the corresponding ovary is exteriorized and removed. Remaining ovarian tissue is re-inserted into the coelomic cavity and checked for excessive hemorrhaging. The surgical wound is closed in two layers. The muscle layer is closed with absorbable suture in a simple interrupted pattern. The skin is closed with monofilament nylon non-absorbable suture in a simple interrupted pattern. The surgical site is monitored daily for 5 days and then every 2-3 days until skin sutures are removed at 10 days.

F. Post-surgical Recovery and Monitoring: It is the general consensus that post-surgical analgesia following oocyte harvesting is not necessary. It is felt that Tricaine (MS-222) may have analgesic properties. After surgery, the animal is allowed to recover for approximately 30-60 min in a container with a level of water not to cover the nostrils of the frog (desiccation of the skin on the dorsum of the frog can be prevented by placing moistened gauze on any exposed surfaces). Once the frog is active and mobile the water level can be raised to a more normal level and the gauze removed. Dechlorinated water is to be used during recovery from anesthesia and the container must be covered to prevent escape attempts. If dechlorinated water is not available, tap water containing 50g/L of non-iodized salt may be used (frogs must not remain in this type of water for longer than 12 hours). Frogs must be monitored daily for at least 5 days after surgery for evidence of excessive inflammation of the incision site, suture dehiscence, or abnormalities indicative of illness (anorexia, listlessness, lethargy, bloating, or “red-leg”). If evidence of wound infection or illness is noted then LARC vet services are to be contacted for evaluation and treatment or the animal shall be euthanized.

Single housing or small-group housing for several days after surgery shall be provided as part of the post-surgical care of laparotomized animals. Frogs shall be monitored daily during the post-operative period (at least 5 days) for the appearance of a normal appetite as well as for any complications such as dehiscence or infection. The appearance of such adverse effects provides justification for immediate euthanasia.

G. Euthanasia: Consist with IACUC Policy 9, Euthanasia, may be achieved either by intracoelomic co-injection of sodium pentobarbital (1100 mg/kg) plus phenytoin (141 mg/kg) solution, or 1100 mg/kg sodium pentobarbital solution (Fatal Plus® is 390 mg/ml sodium pentobarbital) and wait 3 hrs. OR immersion in fresh, buffered (pH 7.0-7.5) Tricaine methanesulfate (tricaine mesylate, Tricaine-S, TMS, MS 222) solution (high dose: 5 - 10g/L) for at least 60 minutes (attended). THEN followed by Decapitation and Pithing or Double-Pithing with training and written IVET permission.
If using MS 222, buffer the MS 222 with sodium bicarbonate (~2:1 weight ratio with MS 222). Check pH. Light sensitive. Make fresh – do not use stored concentrated stock solution. Prepare solutions under a fume hood and wear nitrile gloves, mask, and eye protection. Always use gloves.

Please contact vet services if assistance with the pithing technique is required.

H. Surgical Records: A "Surgical Record" shall be completed immediately after the surgical procedure. Records may be in composite (group) format and can be included as part of research data collected, but shall be available for review.

Records shall identify the individual animal identification, type of surgical procedure performed, the date of the procedure, the person who performed the procedure (or initials), information on drug administration and perioperative monitoring, and shall be maintained in the laboratory. This information shall be available for review by regulatory bodies, including the IACUC.

5. Exceptions
   Any and all deviations from this policy shall be presented to and approved by the IACUC.

References