



Duke University  
Animal Care & Use Program  
Policy



## OOCYTE COLLECTIONS FROM AMPHIBIANS

**PERFORMANCE STANDARD:** All amphibians will be provided appropriate care, and handled in a manner which is humane and supportive of research objectives.

**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 for scientific reasons, considering the lack of complications when performed by competent personnel.

**ROLES:** All participants will adhere to this policy, unless an exception is granted by the IACUC.

**PROTECTION POSTURES REQUIRED:** Gloves will be worn when handling animals and / or animal support equipment.

**POLICY:** The number of laparotomies on frogs to obtain oocytes should be conditional upon the health of an individual animal, quality of the oocytes obtained, the age of the animal, and probable duration of egg production. Multiple recovery surgeries on individual frogs must be scientifically justified and approved by the IACUC.

- Up to a maximum of five recovery surgeries (with a final 6th terminal surgery) will be permitted.
- Waivers may be granted on an exceptional basis by the IACUC for compelling scientific reasons.
- Surgeries must be performed by trained persons using appropriate anesthesia, such as tricaine methane-sulfonate (MS-222).
- Cooling is not acceptable for use as an anesthetic. The 2007 AVMA Guidelines for Euthanasia indicates that “there is no evidence that whole body cooling reduces pain or is clinically efficacious.”
- Surgeries must be done using aseptic technique appropriate for amphibians.
- Single housing for 3 days after surgery should be provided as part of the post surgical care of laparotomized animals.
- Frogs must be monitored daily (and documented daily) during the post-operative period (at least one week) for appetite as well as any complications such as dehiscence or infection. Care will be provided as approved in the animal use protocol.
- Documentation must be provided by the investigator that project personnel have demonstrated competency to perform anesthesia, surgery, and necessary monitoring to the satisfaction of the IACUC and the attending veterinarian or a qualified individual designated by the attending veterinarian.

- Adequate recovery time must be allowed between laparotomies. Investigators should alternate oocyte collection between right and left ovaries and rotate frogs so that the interval between surgeries is maximized. Surgery intervals should not be less than one month, unless waivers are granted by the IACUC for compelling scientific reasons.
- An appendix is attached as a suggested approach.

## APPENDIX

### **Xenopus oocyte harvest recommended protocol:**

- Reference:
  - o Oocyte Harvesting in *Xenopus Laevis*. 2001 revision. ARAC Guidelines, <http://oacu.od.nih.gov/ARAC/index.htm>
  - o Wright, K.M. and B.R. Whitaker. 2001. *Amphibian Medicine and Captive Husbandry*. Krieger Publishing Co. Malabar, Florida.
- Pre-operative considerations: Frogs should be fasted for 12 hours or less to prevent emesis during anesthesia even though associated complications are minimal.
- 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/water). Dosage selection is dependent upon the weight/size of the frog and the duration of anesthesia required. The added benefit of MS-222 use is that this compound has analgesic properties and removes the necessity of administration of post surgical analgesia in most cases. The final solution of MS-222 should be buffered to a pH between 7.0-8.0 by the addition of between 1 to 4 g/L Sodium Bicarbonate (baking soda). This will decrease induction time and reduce anesthetic "excitation" phase. Once a surgical plane of anesthesia has been reached, (noted by a lack of response to deep pain, i.e. toe pinch) frogs should be placed in dorsal recumbency on the non-absorbent "blue" side of a clean/unused diaper pad. 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 anesthetic recovery.
- Aseptic precautions:
  - o Instrument Sterilization: Clean surgical instruments should be wrapped, or packed appropriately before being sterilized with high heat and pressure or high heat alone (steam autoclave or drying oven) or adequate exposure to gas (ethylene oxide). Instruments should be stored in a dry place in which the integrity of the wrapping or packing material will be maintained for a defined period of time. If multiple surgeries are to be done on different animals, then previously sterilized instruments can be re-sterilized using a glass bead sterilizer.
  - o Surgical Skin Preparation: Skin asepsis in *Xenopus* sp. is not typically required for the most common surgical procedures done with this animal. However, the removal of gross debris from the surgical incision site only (via brief rinse with sterile saline, moistened small gauze pad or cotton swab) is recommended. If gloves, a clean lab coat, and sterile instruments are utilized in an aseptic manner and surgery occurs in a dedicated /previously disinfected surgical area on a clean surface; then there is minimal potential for contamination of the surgical site and the development of subsequent post-operative infection. [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 post removal of excess skin secretion from the surgical area. The surgical site should be rinsed with sterile saline before the surgical incision is made. Products that contain soaps or detergents should be avoided and iodine based products must be diluted significantly if they are to be used, i.e. 0.5% betadine solution.]

- **Surgical Procedure:** A small, paramedian, coelomic incision (0.5-2cm) through the skin and muscular layers should be 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 replaced into the coelomic cavity and checked for excessive hemorrhage. Both tissue layers must be closed separately using a monofilament absorbable suture material (3-0 to 4-0) via a simple interrupted suture pattern.
- **Post-surgical Recovery and Monitoring:** Post surgery, *Xenopus* is allowed to recover 4 to 10hrs. 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. Recovery water, de-chlorinated tap water, should contain 50g/L of non-iodized sodium chloride to aid in the recovery from anesthesia (frogs should not remain in this type of water for > 12 hours) and this container should be covered to prevent potential escape attempts. Frogs should be monitored daily for one week post surgery for evidence of excessive inflammation of the incision site, suture dehiscence, or behavioral abnormalities indicative of illness (anorexia, listlessness, or lethargy). If evidence of wound infection or illness is noted then either vet services should be contacted for evaluation and treatment or the animal should be euthanized.
- Exceptions to these guidelines will be considered by the IACUC on a case-by-case basis.