

### **University of Rochester Policy on Major Invasive Surgery (Oocyte Harvest) in Frogs**

The following guidelines were developed by UCAR and the veterinary staff to assist investigators in using frogs in research in accordance with Guide for the Care and Use of Laboratory Animals as well as maximize the quality of oocytes harvested via the surgical approach. AAALAC cites three references listed below describing the importance of aseptic technique for major invasive surgery (e.g. oocyte harvest) in frogs.

1. Multiple survival laparotomies for oocyte harvesting in frogs must be scientifically justified in the UCAR protocol.
2. Frogs experiencing laparotomies must be appropriately anesthetized. The use of hypothermia as an anesthetic is not approved. Transcutaneous anesthesia via immersion in a buffered solution of tricaine methane sulfonate (MS-222) is a common and acceptable anesthetic method in frogs.  
  
MS-222 Anesthetic Protocol:
  - 0.5-2 g/liter MS-222 should be buffered with sodium bicarbonate at 0.42 -1.05 g/liter. (Unbuffered MS-222 solution is irritating to frog skin and poorly absorbed resulting in a prolonged induction time).
  - Surgical anesthesia is achieved within 10-15 minutes. Depth of anesthesia is monitored by lack of a righting reflex, slowed to ceased respiration and loss of response to stimuli.
  - After removal of the frog from the anesthetic solution, maintenance of anesthesia can be achieved by dripping MS-222 anesthetic solution onto the skin.
  - Frogs can be recovered by rinsing with fresh dechlorinated water and/or placement in container of shallow water. Signs of recovery should be evident within 15-30 minutes.
3. Survival surgeries must be performed using modified aseptic technique. This requires the use of a mask, sterile gloves, sterile instruments and materials (e.g., suture) and sterile surgical technique. A sterile prep of the surgical site is usually not indicated for frogs but may be helpful to remove gross surface debris. An appropriate sterile prep for frogs consists of wiping the surgical site with dilute 0.75% chlorhexidine solution or 0.5% povidone iodine solution. The use of soaps or scrubs may be toxic to frogs and is not recommended.
4. Frogs experiencing multiple surgeries must be identified. This can be accomplished by group housing frogs that have experienced an identical number of procedures, and clearly labeling of the housing enclosure. Pattern marking is an alternative identification method which involves recording characteristic skin patterns on each animal.
5. UCAR allows for a maximum of three survival laparotomies with euthanasia at the fourth harvest. A maximum of two surgeries per side is permitted. There should be a period of at least one month between surgeries. Any exemption must be scientifically justified and discussed by the Committee.

**References:**

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- \* Green, S.L. Overview: Factors Affecting Oogenesis in the South African Clawed Frog (*Xenopus laevis*). *Comparative Medicine* 2002 August; 52 (4): 307-312.
- O'Rourke, D.P., Schultz, W.S. (2002) Biology and Diseases of Amphibians. In J.G. Fox, B.J. Cohen, F. M. Loew (eds.), Laboratory Animal Medicine (793-826). New York: Academic Press.
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- \* Schultz, T.W., Dawson, D.A. Housing and Husbandry of *Xenopus* for Oocyte Production. *Lab Animal* 2003 February; 32(2): 34-39.
- \* AAALAC cited