

## **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 to achieve a pH of 6.5-7. (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. Because pain perception in amphibians is analogous to that in mammals, the administration of analgesia is required and may be administered either before surgery when the frog has been anesthetized or during recovery. The following analgesics are acceptable at these recommended doses (Machin 1999, O'Rourke 2008, Terril-Robb 1996):
    - a. Bupivacaine or Lidocaine 0.25% may be swabbed onto the skin or may be infiltrated at the incision site and allowed time to absorb prior to surgery. Do not exceed 5mg/kg total dose either topical or intra-incisional.
    - b. Banamine 25mg/kg IC
    - c. Meloxicam 0.1mg/kg SC SID
    - d. Butorphanol 25mg/kg IC BID
    - e. Xylazine 10mg/kg IC q12-24hrs
    - f. Buprenorphine 14mg/kg via dorsal lymph sac
    - g. Dexmedetomidine 120mg/kg via dorsal lymph sac
  4. Survival surgeries must be performed using modified aseptic technique. This requires the use of a mask, sterile instruments and materials, and sterile surgical technique. Sterile

gloves must be worn unless the investigator utilizes a “tips only” surgical technique. When using “tips only,” non-sterile exam gloves can be worn because the surgeon only uses the sterile tips of instruments for all surgical manipulations. A sterile field must be prepared on which to place instruments regardless of which technique is used. Guidance for sterilization of instrument can be found at <http://www.fda.gov/cdrh/ode/germlab.html>.

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.

Sterile instruments are required regardless of which technique is used. Investigators must indicate their intent to use “tips only” in the protocol. Utilizing the “tips only” technique requires careful attention to detail. Instruments must only be held by the handles, and the tips of instruments must not be allowed to touch non-sterile surfaces. Sutures and other sterile materials to be used in the surgery must only be handled with the instrument tips. Tissues must only be touched with the instrument tips.

If serial surgeries will be performed in one day, instruments (or the tips of instruments if using the “tips only” technique) must be sterilized between animals with the use of a glass bead sterilizer. It is important to remove any gross debris prior to placement of instruments in the sterilizer as well as allowing the instruments to cool sufficiently prior to reuse. Alternatively, sterilized instruments may be kept in a sterile tray containing 70-90% ethyl or isopropyl alcohol for no more than a total of 5 frogs (Keen 2010). The alcohol must be replaced when contaminated with blood or other body fluids. Be sure the alcohol has dried from the instruments before they contact the frog.

5. Post-operatively, frogs must be monitored daily for at least 3 days for appetite as well as any complications such as dehiscence or infection. Such adverse effects may necessitate euthanasia, and the PI should notify DLAM if noted. If non-absorbable suture or wound clips are used, these must be removed no more than 2 weeks after surgery.
6. 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.
7. 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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  9. Species. In D. F. Kohn, S. K. Wixson, W.J. White, G.J. Benson, (eds), Laboratory Animal Medicine (337-378). New York: Academic Press.
  10. Schultz, T.W., Dawson, D.A. Housing and Husbandry of *Xenopus* for Oocyte Production. *Lab Animal* 2003 February; 32(2): 34-39.
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  12. U.S. Food and Drug Administration, (March 2009) FDA-Cleared Sterilants and High Level Disinfectants with General Claims for Processing Reusable Medical and Dental Devices. <http://www.fda.gov/cdrh/ode/germlab.html>