

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

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

1. Multiple survival laparotomies for oocyte harvesting in frogs must be scientifically justified in the approved UCAR protocol.
2. Frogs experiencing laparotomies must be appropriately anesthetized. Transcutaneous anesthesia via immersion in a buffered solution of tricaine methane sulfonate (MS-222) is a common and acceptable anesthetic method in frogs. The use of hypothermia as an anesthetic is NOT approved.

MS-222 Anesthetic Protocol:

- Add sodium bicarbonate at 0.42 -1.05 g/liter to buffer 0.5-2 g/liter MS-222 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. Monitor depth of anesthesia by observation of lack of a righting reflex, slowed to ceased respiration and loss of response to stimuli.
 - After removal of the frog from the anesthetic solution, maintain anesthesia by dripping MS-222 anesthetic solution onto the skin.
 - Rinse frogs with fresh dechlorinated water and/or place them in a container of shallow water to recover from anesthesia. 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 should be administered before surgery (preemptively) when the frog has been anesthetized and continued for a minimum of three days. 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 intracoelomically
 - c. Meloxicam 0.1mg/kg subcutaneously every 24 hours
 - d. Butorphanol 25mg/kg intracoelomically every 12 hours
 - e. Xylazine 10mg/kg intracoelomically every 12-24hrs
 - f. Buprenorphine 14mg/kg via dorsal lymph sac
 - g. Dexmedetomidine 120mg/kg via dorsal lymph sac

Surgeons must use the green "Be Gentle-Post-Op" cage cards to identify post-surgical animals in the vivarium. The cards must contain all information (PI name, procedure/date,

observations, analgesics etc). If an investigator has scientifically justified that analgesics cannot be used pre and post-operatively, it should be noted on the green post-op cards. Remove cards when sutures/wound clips are taken out and maintain with lab notes.

BE GENTLE – POST-OP			
PI _____		ID # _____	UCAR # _____
Procedure _____		Procedure Date _____	
Analgesic _____			
Record observations and analgesic administration as described in the UCAR protocol. Observe rodents at the appropriate analgesic dosing interval following the last treatment to verify that they no longer need analgesics. Remove card from cage at time of suture/wound clip removal and maintain with lab records.			
Date	Time	Observation/Analgesic Administration	Initials

Pain in amphibians may be identified by reduced food intake, lethargy, closed eyes, color change and biting at painful area. Please review the Sedation/Tranquilization, Anesthesia and Analgesia in Laboratory Animals and Veterinarian-Recommended Formularies or contact a DCM veterinarian (X5-2653).

- 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 sterile instruments or “sterile tips” of instruments. 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 DCM 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 in the protocol and approved by UCAR.

References:

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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>

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