



A. PURPOSE

To standardize the surgical harvesting of egg and oocytes in *Xenopus laevis*.

B. GUIDELINES

- The total number of laparotomies for oocyte harvesting must be limited and will depend on the condition of the animal and quality of the oocytes as well as the life span of the animal and duration of egg production. Up to five (5) recovery surgeries (the 6th would be terminal) per animal are acceptable.

Surgery

- Surgeries must be performed by trained personnel using appropriate anesthesia such as tricaine methane-sulfonate (MS-222). MS-222 should have the pH adjusted (5-7) to minimize skin irritation.
- Surgeries must be done as aseptically as practical including the use of sterilized instruments and gloves. Instruments should be sterilized by autoclaving or using a glass-bead sterilizer.
- The use of a cold sterilant should be avoided so that these potentially toxic chemicals are not inadvertently introduced into the surgical site or onto permeable amphibian skin.
- Animals should be placed in clean, fresh water following surgery. As the pain perception of frogs is similar to mammalian species, post-operative pain may occur.
- MS-222, itself, has potential analgesic properties and often can serve as the initial post-operative analgesic agent.
- The post-operative analgesic regimen for frogs should be discussed with a UAC veterinarian. Flunixin meglumine is considered by recent scientific articles to be the most long-acting. However, it is recommended that post-operative analgesia be based on the observation of clinical signs of pain, which include agitation, scratching at the incision site, and change in appetite.
- A trained observer should closely monitor the animals for up to 24 hours post-surgery, with follow up monitoring for 48 hours, to ensure that clinical signs do not indicate a need for additional analgesia.
- The use of these chemical agents may disrupt the normal skin flora of the patient and the constant mucous production of *Xenopus* skin makes any sterilization effort transient. When chemical surgical preps are used, they should be limited to the immediate area around the incision site.
- Careful selection of suture materials and patterns can minimize post-surgical complications. Monofilament sutures such as nylon have been shown to cause less inflammatory reaction in
- *Xenopus* skin. Closure in two layers (muscle layer and skin) is recommended particularly for surgical approaches that are off of the midline. Skin sutures and wound clips, if non-absorbable,



must be removed within 14 days after surgery unless justified for longer duration by consulting veterinarian.

- Post-surgical care of laparotomized animals should include single housing or small group housing for several days. Frogs must be monitored daily during this period for appetite as well as for any complications such as dehiscence or infection. Such adverse effects would be reasons for treatment or euthanasia.
- Adequate recovery time of at least two months must be allowed between laparotomies for oocyte harvest. The investigator may alternate oocyte collection between left and right ovaries and consider rotation of frogs so that the interval between surgeries in any individual is maximized. Scientific justification must be provided for request of recovery times of less than two months which shall be individually reviewed by the IACUC.

Protocol Documentation

- The protocol must include the following information:
 - o The maximum number of laparotomies that will occur for each frog
 - o A justification for the maximum number of laparotomies per frog
 - o The methodology used to identify individual animals or groups of animals to ensure that no frog exceeds the maximum number of surgeries or experiences inadequate recovery time
 - o The analgesic agent(s) that will be used, if needed.

Surgical and Post-Op Records

- All procedures and care must be documented. Records must include at a minimum:
 - o Surgical Records
 - Date of surgery
 - Animal or group identifier
 - Analgesic agent, amount and number of doses provided post operatively
 - o Post-op Records
 - Post-op analgesia (if given) - date administered, animal or group Id, name of drug and dosage
 - Suture removal – Date sutures removed, animal or group ID

C. REFERENCES, MATERIALS, AND/OR ADDITIONAL INFORMATION

Amphibian oocytes are used for studies in molecular biology, embryology and biochemistry. Stage I-VI oocytes are obtained by surgical laparotomy. Multiple surgeries on a single animal may be justified considering the reduction in the total number of animals used over the long term. However, the total number of animals used must be considered relative to the pain or distress experienced by an individual animal.

1. https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/oocyte_harvest.pdf