

COMPARATIVE MEDICINE
LABORATORY ANIMAL FACILITIES

STANDARD OPERATING PROCEDURE
FOR
GUIDELINES FOR OOCYTE HARVEST IN *XENOPUS* FROGS

- 1.0 Purpose:
The purpose of this SOP is to outline procedures for surgical oocyte harvest in *Xenopus* frogs. Stage I-IV *Xenopus* oocytes are often obtained by survival surgical laparotomy. Multiple surgeries on a single animal may be justified considering the simplicity of the procedure, the lack of complications when performed by competent personnel, the effectiveness of anesthetic regimens, and the reduction in the number of animals used if the number of survival surgeries were restricted. The number of laparotomies on frogs to obtain oocytes should be limited only based on the condition and relative health of the individual animal, the quality of the oocytes obtained, the age of the animal and the probable duration of egg production. *Xenopus* frogs may not undergo oocyte harvest laparotomies more frequently than every eight weeks. Frogs should be identified to ensure the appropriate recovery time between survival surgeries.
- 2.0 Scope:
These guidelines apply to all surgical procedures performed on *Xenopus* at the CMLAF in which the animals are expected to recover from anesthesia.
- 3.0 Procedure:
- 3.1 Pre-operative considerations:
- A. Body condition of frogs should be assessed prior to surgery. Only frogs in good body condition should have oocyte harvest performed.
 - B. Frogs should be fasted for 6-12 hours prior to surgery to prevent vomiting.
 - C. Although a dedicated surgical facility is not necessary, the surgical area must be a portion of the room that can be easily sanitized. The surface should be clean, uncluttered, and free from overhanging objects and chemicals. The area should be wiped down with a disinfectant (e.g., 10% bleach, Alcide, Lysol, Staphene, Cidex) before and after use.
- 3.2 Instrument sterilization:
- A. Instruments must be sterilized before use in survival surgery.
 - B. A sterile wrap must be provided as an aseptic surface on which to place instruments during the procedure.
 - C. Acceptable sterilization includes: autoclaving or gas sterilization (ethylene oxide).
 - D. Alcohol is not an acceptable sterilant.
- 3.3 Anesthesia and supportive care during surgery:

- A. All frogs must receive appropriate anesthesia. Tricaine methane sulfonate (MS222) is the anesthetic of choice for *Xenopus*.
- B. Caution should be exercised when handling crystalline MS222, to prevent inhalation and eye and skin exposure.
- C. Dissolve the powder in dechlorinated water in an induction tank at a dose of 500mg-2gr of MS222/L of water.
 - I. MS222 should be buffered to a pH of 7.4. (e.g. addition of 10-25 mEq/L (420-1050 mg/L of NaHCO₃). The pH of the solution should be checked before each use.
- D. Alternatively, the MS222 can be injected subcutaneously, at dose of ~86 mg/kg (50-150 mg/kg).
- E. Place frog in an induction tank shallow enough to ensure that the frog will not drown.
- F. Ensure temperature of the solution is the same as ambient temperature for the frog to avoid shock.
- G. The working solution must be prepared fresh each day.
- I. There is no evidence demonstrating appropriate analgesia with hypothermia in amphibians, although the animals become torpid. Direct contact with skin can cause burns. Thus, ice or ice water is not acceptable as an adjunct to anesthesia and should not be used.
- J. Induction takes approximately 15 minutes. Depth of anesthesia can be monitored through loss of the righting reflex and loss of response to painful stimuli.
- K. Once the animal is anesthetized, remove it from the solution and place the frog on the non-absorbent (blue) side of a clean/unused under pad.
- L. Keep the skin moist.
- M. Anesthesia should persist for approximately 10-20 minutes.
- N. A diluted (50%) solution of anesthetic solution can be dripped on the frog skin, or a paper towel soaked in MS222 can be placed underneath the frog's back, should longer anesthesia be needed.
- O. Alternatively, 1/3 of the calculated dose can be injected SC for maintenance.
- P. Care should be taken not to drip anesthetic solution into the surgical incision, as this prolongs recovery.

3.4 Surgical procedure:

- A. Aseptic technique must be used whenever harvesting oocytes surgically.
- B. A clean lab coat or scrub suit, mask and **powder free** sterile gloves should be used. Gloves should be changed between frogs.
- C. *Xenopus* skin contains antimicrobial agents. Skin prep is not always necessary, although gross debris should be removed by gently flushing with diluted chlorhexidine solution.
- D. Soaps and scrubs should not be used, as they will disrupt the protective mucous layer on the skin.

- E. A small, paramedian, coelomic incision (0.5-2cm) through the skin and muscle layers should be made on either the right or left side of the abdomen.
- F. A portion of the corresponding ovary is exteriorized with forceps and the desired amount of oocytes is removed.
- G. Remaining ovarian tissue is replaced into the coelomic cavity and checked for excessive hemorrhage.
- H. Both tissue layers must be closed separately using a monofilament suture (3-0 or 4-0) in a simple interrupted pattern. The muscle layer should be closed with absorbable suture (e.g. vicryl), the skin with non-absorbable (e.g. silk).

3.5 Analgesics (Pain Relief):

- A. All frogs should receive at least one dose of post-operative analgesia. Acceptable analgesics include: flunixin meglumine (Banamine) (25 mg/kg intracoelomic once or 25 ug/gm BW q 300 hrs.) and xylazine (10 mg/kg intracoelomic q 12-24 hrs).
- B. Analgesics can be administered pre-operatively, ~15 minutes prior to MS222 administration, however, the dose of MS222 may need to be reduced.

3.6 Post-surgical recovery and monitoring:

- A. After surgery, the frog should be gently placed in dechlorinated frog water in a shallow recovery container with a level of water that does not cover the nostrils of the frog (to reduce risk of drowning).
- B. Once the frog is active and mobile, it can be placed in a tank by itself or with a small number of post-operative frogs.
- C. Frogs should be monitored daily for 2 days after surgery for evidence of inflammation at the incision site, suture dehiscence, infection or behavioral abnormalities indicative of illness (anorexia, listlessness, bloating).
- D. If evidence of wound infection or illness is noted then either CMLAF vet services should be contacted for evaluation and treatment, or the animal should be euthanized.
- E. Sutures should be removed in 6 weeks if they have not sloughed off with shed skin.
- F. Frogs should be rested for at least 8 weeks between oocyte harvests, and the side of oocyte harvest should be alternated to allow maximal healing time.

3.7 Surgical records:

- A. A surgical record must be completed immediately after the surgical procedure (see Attachment).
- B. Records may be in composite (group) format.
- C. Records must identify the type of surgical procedure performed, the date and duration of the procedure, the person who performed

the procedure, anesthetic used, post-operative analgesia and post-operative monitoring (see attachment).

- D. The surgical record must be submitted to the veterinary staff (BEB Room 204) at the completion of surgery. The CMLAF veterinary technicians will monitor frogs post-operatively for a minimum of two days.

3.8 Training:

- A. Professional and technical personnel and students who perform anesthesia and surgery must be trained to accomplish these tasks in a humane and scientifically acceptable manner.
- B. The principal investigator must assure the IACUC that the project personnel have demonstrated competency or will be adequately trained to perform anesthesia and surgery.
- C. The CMLAF veterinary staff is available to provide assistance with, or training in, aseptic and surgical techniques, and the proper administration of anesthesia.