

OOCYTE COLLECTION FROM XENOPUS LAEVIS FROGS
IACUC Standard Procedure
Effective Date: October 2022

Description of procedure:

Oocytes may be harvested up to 4 times per animal, alternating ovaries for each procedure. These procedures must be performed at least four weeks apart, with the last harvest being a terminal procedure. Surgeries must be performed by trained personnel.

Pre-procedural preparation and anesthesia:

- Clean, nonpowdered gloves (Nitrile or Vinyl - no latex) moistened with water must be worn at all times when handling frogs. All surgical procedures must be recorded, and records checked to assure that the frog is an appropriate surgical candidate. Choose a female frog that is large and active. Collect the frog with a net that is dedicated to the tank. Transport frogs in water from their home tank or in same temperature DI water from the central faucet to and from the lab.
- Anesthetize the frog with a fresh mixture of Tricaine methane sulfonate (MS222). Cooling and hypothermia are not recommended as an adjunct to MS-222 anesthesia. The solution must be buffered to a neutral pH of 7.0. As MS222 is a respiratory irritant, it must be prepared in a chemical fume hood. Users needing respirators should contact EH&S for fit-testing and medical clearance prior to use.
- Place the frog in a shallow bath of MS222 with nostrils above the level of the anesthetic bath and wait for the onset of anesthesia - it usually takes approximately 15-20 minutes.
- Confirm a surgical level of anesthesia by gently pinching the fleshy part of both rear feet with hemostats or forceps and ensuring that the frog is non-responsive to painful stimuli.
- Place the frog on a surface of several clean paper towels wetted with the MS222 bath solution.
- Remove contaminants on the skin by gently rinsing for 5 seconds with a steady stream of sterile 0.9% saline at only the portion of the skin where the incision will be made. Do not use alcohol, chlorhexidine or iodine as these agents are not recommended for use in amphibians. Do not use gauze, which is too abrasive.

Oocyte collection:

- ***The use of sterile instruments is required for survival collection surgeries.*** If you will be touching the surgical site with your gloved fingers, the gloves must be sterile. Sterilize instruments by autoclaving or using a glass bead sterilizer. If a bead sterilizer is used, allow instrument tips to cool completely before touching tissues. Cold sterilization fluids (such as cidex or zephirin) should be avoided, as they may introduce potentially toxic chemicals into the surgical site or onto permeable amphibian skin.
- Make a small incision (1-2cm horizontal or vertical) on the abdomen above the groin and in between the midline and the lateral aspect of the abdomen.
- Use blunt scissors to dissect through the fascia and muscle to visualize the oocytes. Gently externalize oocyte strands and remove oocytes. Close the incision in two layers by suturing

the fascia and skin layers separately. Swab the incision site with 0.25% bupivacaine (0.5-1.0 ml) before suturing.

- Skin should be closed using an interrupted suture pattern to prevent dehiscence. Monofilament suture material (not braided) must be used. Use 4-0 to 5-0 polydioxinone (PDS) to close the fascia and 4-0 PDS or nylon to close the skin layer.
- Recover the frog in clean tank water with head elevated (to prevent drowning) and the rest of the body submerged. Recovery takes up to 1 hour. Monitor frequently, at least once every 10 minutes.

Post-collection care:

- Frogs may be returned to a separate recovery tank in standard housing when animals are able to swim normally.
- Monitor frogs daily for a minimum of 48 hours for wound dehiscence or infection and then verify overall mobility, appearance and muscle tone every 2-3 days for two weeks. Contact LARC veterinary staff if any health concerns arise.
- Remove skin sutures 14-21 days after procedure if nylon is used

Identification, tracking and record keeping:

- If the rationale for performing MPOX (multiple partial ovariectomies on xenopus) is to identify and re-use individual frogs known to produce optimal oocytes, the investigator must provide a plan for:
 - Tracking these frogs;
 - Tracking the number of surgeries performed on each frog;
 - Ensuring the minimum interval of 4 weeks between surgeries.
- Procedures for tracking can include segregation of operated animals into different post-surgical holding tanks or physical marking of individual animals. Physical identification may include web notching, suture and/or glass beads in the foot web, or elastomer tags. The identification method must be described in the approved protocol.

Documentation required:

- Identification or tracking method used and location on the animal if applicable.
- Name of the individual conducting the procedure.
- Date and type of surgical procedure, number of surgeries performed, and applicable comments (e.g. frog recovered normally from anesthesia).
- Anesthetic agent used.
- Analgesics provided.
- Post procedure monitoring.
- Date of suture removal applicable.
- Euthanasia.
- Records must be readily available for review during program inspections by LARC, the IACUC or outside regulatory bodies.

Agents:

The procedure requires anesthetic MS-222. Analgesic (0.25% bupivacaine) is required. All agents must be listed in the appropriate protocol section.

Potential adverse effects to be considered:

Infection of surgical site, poor wound healing, dehiscence, decreased appetite.

References:

1. Boston University, Guidelines for *Xenopus* Surgical Oocyte Harvest
2. Elsner et al. Poor quality of oocytes from *Xenopus laevis* used in laboratory experiments: prevention by use of antiseptic surgical technique and antibiotic supplementation. [Comp Med](#). 2000 Apr;50(2):206-11
3. Green SL. Overview: Factors affecting oogenesis in the South African Clawed Frog (*Xenopus laevis*). *Comp Med* 2002, 52:307-312
4. Guide for the Care and Use of Laboratory Animals
5. NIH Guidelines for Egg and Oocyte Harvesting in *Xenopus laevis*
6. University of California, Berkeley Multiple Partial Ovariectomies on *Xenopus* Policy
7. Tuttle et al. Evaluation of the Gross and Histologic Reactions to Five Commonly Used Suture Materials in the Skin of the African Clawed Frog (*Xenopus laevis*). [Journal of the American Association for Laboratory Animal Science](#), Volume 45, Number 6, November 2006, pp. 22-26(5)
8. Wright and Whitaker, Amphibian Medicine and Captive Husbandry
9. Philips BH et al. Evaluation of Presurgical Skin Preparation Agents in African Clawed Frogs (*Xenopus laevis*). *JAALAS* 2015 Bivl 54(6): 788-98
10. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition, p. 94