



## **GUIDELINES FOR OOCYTE HARVESTING FROM FROGS**

Amphibian eggs and oocytes such as those from *Xenopus* spp (*X. laevis* and *X. tropicalis*) are often used for studies in molecular biology, embryology and biochemistry. Induction of ovulation and/or gentle squeezing of females is used to collect unfertilized eggs while stage I-VI oocytes (immature eggs not capable of being fertilized) are collected using a surgical laparotomy approach. It should be noted that commercially available *Xenopus* oocytes may be a desirable alternative to surgical harvesting if possible.

Multiple survival surgeries on a single animal are discouraged, but may be approved with scientific justification. IACUC recognizes that multiple oocyte harvests from an individual frog may reduce the total number of animals used but could enhance the quality of the research by allowing multiple collections from animals that produce high quality oocytes. However, the reduction in the number of animals used must be balanced with the need to minimize the pain and distress experienced by individual frogs. The IACUC has the following guidelines regarding the harvest of *Xenopus* oocytes by surgical laparotomy:

1. Surgeries must be performed by trained personnel using appropriate anesthesia. Surgeries should be done as aseptically as possible, including the use of sterilized instruments and powder-free gloves. Instruments should initially be sterilized by autoclaving and by use of a glass-bead sterilizer intraoperatively. The use of cold sterilants is not recommended for instruments that will be used on amphibians.
2. Animals must be at a surgical plane of anesthesia (loss of righting reflex, loss of withdrawal reflex to toe pinch) before the procedure begins. To maintain anesthesia, the frog can be exposed to water containing dissolved Finquel/Tricaine. Care should be taken to ensure that anesthetic-laced water is not introduced into the incision, as this may prolong recovery. Animals must never be left unattended during surgery and should be periodically rinsed with water to avoid drying out their skin.
3. The technique for skin preparation and use of surgical drapes remains controversial for aquatic species and these are not recommended for *Xenopus* to avoid disrupting the delicate mucous layer that serves protective functions. Generally, chemical surgical skin preps should be avoided since they could be systemically absorbed and adversely affect the frog. Chlorhexidine is not permitted nor scrubs containing soaps or detergents. Currently, rinsing with a steady stream of 0.9% sodium chloride alone or 0.5% povidone iodine (with a final rinse of 0.9% saline) for at least 5 seconds is recommended for the preparation of the surgical site.
4. Suture materials are important and monofilament (nylon) is recommended since it causes less complications than other materials in *Xenopus*. Sutures should be removed after 2 weeks if they have not been sloughed by this time.
5. Recovery from anesthesia may be prolonged. It is essential that the frog be positioned in a tank of shallow conditioned and de-chlorinated water with the body submerged but the head elevated to avoid drowning. Animals should be observed until they fully recover from surgery prior to returning them to the housing room.
6. Analgesia use in frogs after surgery remains a topic on which little definitive information is available. Some analgesics used in other species have unclear efficacy in frogs. In animals exhibiting a loss of appetite, administration of flunixin meglumine (25 mg/kg) by injection into the dorsal lymph sac may be helpful and should be discussed with the veterinary staff.

### **Limitations on the Number and Frequency of Surgeries:**

- The number of laparotomies should be limited, depending on the condition of the animal. Based on current practices in the field, a maximum number of surgeries allowed per individual animal is limited to **3 survival surgeries and one terminal procedure**.
- Adequate recovery time should be allowed between laparotomies. The interval between survival procedures should be **no less than one month** to allow for recovery and healing (and longer times are preferable).
- Each subsequent surgery should be performed on the contralateral side such that oocyte collection alternates between left and right ovaries. Evidence of surgery-related stress such as poor body condition, poor oocyte quantity or quality, and/or clinical disease will require more extended rest periods based on recommendations from the veterinary staff.
- The investigator should rotate frogs so that the interval between egg collections is maximized. Frogs should be individually identified or placed into a formal rotation system that prevents animals from being reused more frequently. Cage cards must have surgery dates indicated; beads sewn onto the skin can be used to identify individuals if frogs are group-housed.
- Single housing or small group-housing for several days to a week after surgery should be considered as part of the post-surgical care of laparotomized animals. Frogs should be monitored daily during this period to ensure they are eating properly, as well as to detect any complications, such as dehiscence or infection. Such adverse effects would be reasons for immediate euthanasia.

### **Information on Anesthesia in Frogs:**

Finquel® / MS-222® (Tricaine methanesulfonate) is a commonly used anesthetic agent for amphibians and it is FDA approved. If an investigator wishes to use a non-pharmaceutical grade chemical in frogs, scientific justification must be provided. The IACUC guideline entitled “Use Of Non-Pharmaceutical-Grade Chemicals And Other Substances In Research Animals” should be consulted for additional information.

*NOTE: Finquel® / MS-222® / Tricaine should NOT be disposed by dumping down the drain since it is known to be problematic to the environment. IACUC requires disposal of this material as a biohazard and PIs must have an approved SOP*

### **Non-surgical Harvesting of Unfertilized (Stage 0) *Xenopus* Eggs:**

This method (also known as “milking” or stripping) involves an initial injection of human chorionic gonadotropin (HCG) into the dorsal lymph sac of female animals. Animals may receive a single dose of 500-800 IU HCG (1000 U/ml) injected into the dorsal lymph sac, or are primed using 0.5ml of 200 U/ml PMSG followed by 0.5ml of 1000U/ml HCG 2-3 days later. Females should begin laying eggs 12 -14 hours after the HCG injection and the harvesting process involves gently squeezing eggs from females around that interval. When properly performed by trained research personnel, female *Xenopus* are not harmed by the egg harvesting procedure and can be used again after a recovery period of 3-6 months. Animals should be used for this procedure no more than 4 times.

### **Reference:**

Guidelines for Egg and Oocyte Harvesting in *Xenopus* Species

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