The University of Pennsylvania’s Institutional Animal Care and Use Committee (IACUC) has adopted the following guidelines for egg and oocyte harvesting from laboratory frogs (*Xenopus Laevis*). These guidelines are based on the National Institutes of Health Intramural "Guidelines for Egg and Oocyte Harvesting in *Xenopus laevis*" (NIH).

**Background**

Removing oocytes/eggs from *Xenopus laevis* requires either a non-surgical procedure (often known as “milking”) or an abdominal surgical procedure. The method chosen depends on the stage of oocyte needed and the research objectives [1,2].

“Milking” provides mature eggs that are ready for in vitro fertilization. This procedure involves applying gentle pressure with the hand to the ventral and lateral sides of the abdomen of females that have been hormonally primed 12 hours prior (injected with 500 IU of HCG into the dorsal lymph sac). The female may undergo this procedure multiple times, with a recovery period of 3-6 months between procedures. To avoid causing stress or harm to the frog, this procedure should only be performed by those individuals trained and experienced in performing the procedure.

The surgical method is most appropriate to collect stage I-VI oocytes. It is possible to surgically remove oocytes from one frog multiple times, though deterioration in oocyte quality is sometimes seen after multiple surgeries. Multiple harvest surgeries can occur without significant morbidity and mortality when trained investigators use proper surgical techniques.

The University of Pennsylvania limits the number of surgical oocyte harvesting procedures in any one frog to five (5) survival surgeries followed by a terminal surgery provided that time between surgeries is at least 8 weeks and that the procedure will only be performed if the animal is physically normal and completely recovered from the previous surgery. Alternate sides of the abdomen should be used for successive laparotomy procedures. A reliable method of identification or an organized rotational system, as well as accurate record keeping, is required in order to ensure that animals are not subjected to more than the approved number of surgical procedures.

**Guidelines**

1. Aseptic Procedures for Surgical Method

   **A. Surgical area**

   1. Although a dedicated facility is not necessary, the surgical area should be a dedicated space that is easily sanitized, uncluttered and away from high traffic flow to minimize contamination from other laboratory activities.
2. The work surface must be thoroughly cleaned with an approved hard surface disinfectant (e.g. Clidox®, 10% sodium hypochlorite, 70% alcohol) prior to and after each procedure.

B. Instruments

1. All instruments must be cleaned and sterilized prior to use on animals. Instruments should be sterilized by autoclave or glass-bead sterilizer. The use of cold sterilants should be avoided due to the possibility of introducing these chemicals onto the permeable amphibian skin.

2. Ensure selected tools are appropriate for surgery and worn or rusted surgical instruments or those not manufactured for surgical use are not permitted.

3. The suture material chosen should be carefully considered. Monofilament nylon is the preferred suture material when working with *Xenopus*. Other suture materials have been shown to cause local inflammatory reactions [3].

C. Animal

1. The recommended preparation method for the surgical incision site is gentle removal of gross debris followed by thorough rinsing with sterile saline.

2. The uses of surgical drapes and more intense preparation of the surgical site remain controversial for aquatic species. Some publications recommend a sterile drape and site preparation with dilute povidone iodine or chlorhexidine solution [4], while others suggest that these may disrupt the normal skin flora of the frog due to the semi-permeable nature of the skin [5].

3. Currently the University of Pennsylvania does not recommend preparation of the surgical site with anything other than sterile .9% saline and .5% Povidone Iodine. Chlorhexidine is not permitted [6].

4. The Principal Investigator should make decisions regarding surgical site preparation method in consultation with ULAR veterinary staff.

D. Surgeon

1. Perform appropriate surgical scrub.

2. Don a clean lab coat, scrubs, or other appropriate clothing and clean powder-free gloves.

3. Gloves must be changed between animals.

4. Movements of the surgeon and assistant(s) should be done carefully to avoid contamination of the surgical site.

2. Anesthetics

A. Surgery should be performed using appropriate anesthesia such as immersion in a 1-2 grams/liter solution of tricaine methane sulfonate (MS-222). A fresh solution of MS-222
must be made daily and should be buffered to a neutral pH of 7.0-7.5 using sodium bicarbonate. Other anesthetic agents may also be acceptable and should be chosen based on investigator experience and consultation with a ULAR veterinarian.

B. Animals must be at a surgical plane of anesthesia before a surgical procedure begins. An appropriate plane of anesthesia is indicated by loss of the righting and withdrawal reflexes. If at any time an animal responds to a toe pinch or to any procedural stimulus, stop the procedure and give a supplemental dose of anesthetic.

C. Care should be taken to ensure anesthetic water is not introduced into the incision, as this may prolong recovery.

D. Hypothermia alone is inadequate for maintaining a surgical plane of anesthesia.

E. During a procedure, animals that are under anesthesia should NEVER be left alone.

F. Frogs should be periodically rinsed with water to avoid drying out of the skin.

3. Post-operative Care

A. Recovery from anesthesia may be prolonged. Place the frog in a shallow tank of fresh water with the body submerged and head elevated to prevent drowning. Animals should be observed until they recover from anesthesia and observations should be recorded at least every 15 minutes. The frogs should be evaluated for general appearance, muscle tone and mobility.

B. Frogs cannot be returned to the housing room until fully recovered from anesthesia and they should be housed individually or in small groups for several days. They should be monitored daily for a minimum of one week noting any changes in appetite and any complications, including dehiscence or infection at the incision site. Any signs of illness, abnormal behavior, or pain must be reported promptly to a ULAR veterinarian.

C. There is a paucity of information regarding the appropriate use and dosing of analgesics in *Xenopus laevis* [7]. There is some evidence that analgesics commonly used in other species or for other applications may have limited efficacy in frogs following the oocyte harvest procedure. A handful of studies have shown that administration of Flunixin meglumine (25 mg/kg via the dorsal lymph sac) results in analgesia in *Xenopus* and other frog species [8]. Meloxicam (0.1 mg/kg IM once daily) has also been shown to provide analgesia in other species of frogs [9]. Neither drug has been associated with negative side effects.

4. Record Keeping

All records should include: date, animal ID and location (ie: tank number), protocol number, and lab personnel’s initials. The following specific records should be available upon request for inspection:

A. Surgical/procedural records- These should also include a brief description of the procedure, route and dose of anesthesia, and any deviations from the approved protocol.
B. Post-procedure records—These should also include all treatments (doses and routes of administration), and a comment on the general condition and health of animal (activity level, condition of incision, etc.).

References