IACUC Guideline on Egg and Oocyte Harvesting From *Xenopus Laevis*

**I. Background**

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

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 providing that time between surgeries will be at least four (4) weeks and that the procedure will only be done if the animal is physically normal, with complete healing from the previous surgery.**

The “milking” procedure 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 belly of hormonally primed females to expel eggs. The female may undergo this procedure multiple times, with a recovery period of 2-4 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 the procedure.

**II. Aseptic Procedures for Surgical Method**

A. **Location/Field**

1. Although a dedicated facility is not necessary, the surgical area should be a dedicated space that is easily sanitized, uncluttered and away from the flow of traffic 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. Sterilization methods include steam, ethylene oxide, and dry heat (alcohol is NOT a sterilant). A glass bead sterilizer is recommended to sterilize instruments between animals.

2. Ensure that the tools are appropriate for surgery (i.e. do not use worn or rusted surgical instruments or those not manufactured for surgical use).

C. **Animal**

1. At a minimum, a brief rinse with sterile saline to remove gross debris from the surgical incision site is recommended.

2. The use of surgical drapes and preparation of the surgical site remains controversial for aquatic species. Some publications have recommended a sterile drape and site preparation with dilute povidone iodine or chlorhexidine solution\(^3\), while others suggest that these may disrupt the normal skin flora of
the animal due to the nature of their semi-permeable skin. When chemical surgical preps are used, they should be limited to the immediate area around the surgical site. Please determine animal preparation method in consultation with ULAR veterinary staff.

D. Surgeon
   1. Perform surgical scrub.
   2. Wear a clean lab coat, scrubs, or other appropriate gown.
   3. Wear clean powder-free gloves changed between animals.
   4. Movements of the surgeon and assistant(s) should be done carefully to avoid contamination of the surgical site.

III. Anesthetics
   A. Frogs are typically anesthetized by immersion in a 0.15% solution of tricaine methane sulfonate (MS-222). A fresh solution of MS-222 must be made daily.
   B. Animals must be under surgical plane of anesthesia before a surgical procedure begins. Check animal prior to and during the procedure using toe pinch. If at any time an animal begins to come out of the anesthetic effects stop the procedure and give a supplemental dose of anesthetic.
   C. To maintain level of anesthesia, the frog can be exposed to water containing dissolved MS-222. Care should be taken to ensure anesthetic water is not introduced into the incision, as this may prolong recovery. Frogs may also be placed on ice after they are adequately anesthetized to slow the blood flow. The animal should be placed on a towel or pad and the animal should NEVER be in direct contact with the ice. Hypothermia alone is inadequate for maintaining a surgical plane of anesthesia.
   D. During a procedure, animals that are under anesthesia should NEVER be left alone.
   E. Frogs should be periodically rinsed with water to avoid drying out of the skin.

IV. Post-operative Care
   A. Recovery from the anesthesia can take up to an hour. To hasten the recovery process, rinse the frog with fresh water, placing it in shallow water as it may drown if unconscious. Animals should be observed until they recover from anesthesia and records of this observation should be made 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 for appetite and any complications, including dehiscence or infection. Should an animal demonstrate signs of ill health, abnormal behavior, or pain it must be euthanized or put on a treatment plan developed in association with a ULAR veterinarian.

V. Record Keeping
   The following records should be available upon request for inspection. Associated with this guideline is a template of a procedure and post-procedure monitoring sheet:
A. Surgical/procedural records - These should include the protocol number, animal identification/tank number, date of surgery/procedure, lab personnel’s initials, method of anesthesia, brief description of procedure, and any deviations from the approved procedure.

B. Post-procedure records - These should include the protocol number, animal identification/tank number, initials of individual making observations, date(s) of observation(s), any treatments provided, and a comment on the general condition and health of animal.

VI. References