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**1. PURPOSE**

The intent of this Standard Operating Procedure (SOP) is to describe procedures for the collection of eggs and oocytes in *Xenopus laevis*.

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**2. RESPONSIBILITY**

Principal investigators (PI) and their staff, veterinary care staff or any individual performing surgery on frogs, or assisting in those procedures.

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**3. MATERIALS**

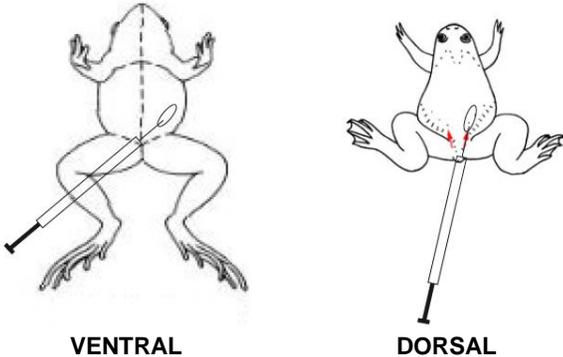
- 3.1. Powder-free, non-textured gloves
- 3.2. Anesthetics
- 3.3. Human chorionic gonadotropin (HCG)
- 3.4. Pregnant mare serum gonadotropin (PMSG)
- 3.5. 0.9 % Saline
- 3.6. Sterile surgical instruments
- 3.7. Sterile cotton-tipped swabs
- 3.8. Suture material (monofilament nylon, polydioxanone or polyglactin 910)

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**4. PROCEDURES**

- 4.1. Moisten gloves with tank water prior to handling frogs.
- 4.2. Hormone induced unfertilized egg collection (non-surgical):
  - 4.2.1. Females may be used repeatedly for egg harvesting as long as they are in good health.
  - 4.2.2. Allow for a 3 month recovery period between egg harvesting procedures.
  - 4.2.3. Anesthesia is not required.
  - 4.2.4. Preparation of hormones:
    - 4.2.4.1. PMSG: prepare a 1IU/ml solution by adding 2000IU to 2mL 0.9% saline.
    - 4.2.4.2. HCG: prepare a 500IU/mL solution by adding 2500IU to 5 mL of 0.9% saline.
    - 4.2.4.3. Store hormone solutions at 4°C for up to three months.
  - 4.2.5. Procedure:
    - Day 1:** Prepare tank for female.  
Administer 50µl (50UI) PMSG into female frog. The PMSG can be injected intraperitoneally into the ventral side of the frog or into the lymph sac on the dorsal side (see figure 1).  
Squeeze the frogs firmly in the net to avoid their moving. Make sure to hold the syringe at an angle nearly parallel to the skin.
    - Day 2:** Clean female's tank by exchanging 90% of the water with fresh water from the system.
    - Day 4 AM:** Administer 150 UI (0.3ml) of HCG by injecting into the dorsal lymph sac of the male.
    - Day 4 PM** (around 4pm) - Make sure that tank of female is clean, if not exchange 90% of water.  
Administer 150 UI (0.3ml) HCG to the male and 400UI HCG (0.8ml) to the female and place animals together in tank.  
If the two animals are not in amplexus one day after injection, wait another day or repeat the Day 4 injections.

Figure 1: animal restraint and intraperitoneal injection



**Day 5:** Females should begin laying eggs 12 hours after HCG injection.

- 4.2.6. Eggs are obtained either by allowing the female to lay the eggs in a tank or by gently massaging the abdomen and sides of the frogs (egg stripping).
- 4.2.7. Monitor animals for appetite and general condition after each procedure.
- 4.3. Surgical Oocyte collection:
  - 4.3.1. A maximum of 4 surgical procedures is allowed with 3 being survival procedures and the fourth being terminal.
  - 4.3.2. Alternate between the right and left ovaries for each oocyte collection procedure.
  - 4.3.3. Allow for a 2 month recovery period between surgical procedures.
  - 4.3.4. Frogs must not be fed on the day of surgery to avoid emesis while anesthetized.
  - 4.3.5. All post-surgical animals must be appropriately identified.
  - 4.3.6. Surgeon's preparation:
    - 4.3.6.4. Wash hands.
    - 4.3.6.5. Wear a surgical mask and a clean lab coat.
    - 4.3.6.6. Use aseptic technique.
    - 4.3.6.7. The surgeon must avoid touching non-sterile surfaces during the procedure.
  - 4.3.7. Surgical Principles/Aseptic technique:
    - 4.3.7.1. Designate a sterile area on the working surface for the sterile material (instruments, suture material, gauze, etc.).
    - 4.3.7.2. Begin surgery with clean and sterile surgical instruments, handle them aseptically. Sterilize instruments by autoclaving or by using a glass bead sterilizer. Avoid liquid cold sterilization solutions as these chemicals should not come in contact with the frog's permeable skin.
    - 4.3.7.3. Disinfect the instruments between each animal by dipping them in a hot glass bead sterilizer for approximately 30 seconds after removing any blood and debris (let cool completely).
    - 4.3.7.4. Dip suture material in 70% alcohol between each animal.
  - 4.3.8. Procedure:
    - 4.3.8.1. Anesthetize animal as per Fish and Aquatic Amphibian SOP. Hypothermia is not recommended as an adjunct to anesthesia as it will prolong recovery.
    - 4.3.8.2. Prior to surgery, verify depth of anesthesia by loss of animal's pedal withdrawal (toe pinch) reflex.
    - 4.3.8.3. Keep the skin moistened during surgery.
    - 4.3.8.4. Contaminants on the skin can be gently removed using a cotton-tipped swab. Do not apply alcohol or other preparations that contain alcohol directly to the skin of an amphibian as absorption of these products through the skin may dissolve normal secretions that protect the animal from dehydration and infections.
    - 4.3.8.5. Make a small incision (1-1.5cm) on the abdomen above the groin, lateral to the midline.
    - 4.3.8.6. Use blunt scissors to dissect through the fascia and muscle to visualize the oocytes.
    - 4.3.8.7. Oocyte strands are then gently externalized and cut.
    - 4.3.8.8. One stitch using absorbable suture may be taken in the ovary to control bleeding.
    - 4.3.8.9. After ensuring that there is no hemorrhaging from the surgical site, the abdominal muscles and skin layers can be sutured together as a single layer or in two separate layers.
    - 4.3.8.10. Skin sutures must be non-absorbable, preferably monofilament nylon, and removed 10-14 days post-operatively.

4.3.9. Post-operative Care:

- 4.3.9.1. Recovery from the anesthesia can take up to an hour. To hasten the recovery process, rinse the frog with fresh aquarium water.
- 4.3.9.2. Place animals in shallow water until fully recovered. During this time check the frogs for general appearance, muscle tone and mobility every 10-15 minutes.
- 4.3.9.3. Frogs should be monitored daily for two weeks for appetite as well as for any complications such as dehiscence or infection of the surgical site

**5. REFERENCES**

- 5.1. Tuttle AD, Law JM, Harms CA, Lewbart GA, Harvey SB. "Evaluation of the gross and histologic reactions to five commonly used suture materials in the skin of the African clawed frog (*Xenopus laevis*)". *J Am Assoc Lab Anim Sci.* 2006 Nov;45(6):22-6.

**SOP REVISION HISTORY**

DATE	PREVIOUS VERSION	NEW VERSION
2016.03.16	4.2.2. Allow for a 4-6 month recovery period between egg harvesting procedures.	4.2.2. Allow for a <del>4-6</del> 3-month recovery period between egg harvesting procedures.
2016.03.16	4.2.4. Inject 500IU of HCG into the dorsal lymph sac.	<p>4.2.4. <del>Inject 500IU of HCG into the dorsal lymph sac.</del></p> <p>4.2.4. Preparation of hormones:</p> <p>4.2.4.1. PMSG: prepare a 1IU/ml solution by adding 2000IU to 2mL 0.9% saline.</p> <p>4.2.4.2. HCG: prepare a 500IU/mL solution by adding 2500IU to 5 mL of 0.9% saline.</p> <p>4.2.4.3. Store hormone solutions at 4°C for up to three months.</p> <p>4.2.5. Procedure:</p> <p>Day 1: Prepare tank for female.</p> <p>Administer 50µl (50UI) PMSG into female frog. The PMSG can be injected intraperitoneally into the ventral side of the frog or into the lymph sac on the dorsal side (see figure 1). Squeeze the frogs firmly in the net to avoid their moving. Make sure to hold the syringe at an angle nearly parallel to the skin.</p> <p>Day 2: Clean female's tank by exchanging 90% of the water with fresh water from the system.</p> <p>Day 4 AM: Administer 150 UI (0.3ml) of HCG by injecting into the dorsal lymph sac of the male.</p> <p>Day 4 PM (around 4pm) - Make sure that tank of female is clean, if not exchange 90% of water.</p> <p>Administer 150 UI (0.3ml) HCG to the male and 400UI HCG (0.8ml) to the female and place animals together in tank.</p> <p>If the two animals are not in amplexus one day after injection, wait another day or repeat the Day 4 injections.</p>