



STANDARD OPERATING PROCEDURE 302 XENOPUS OOCYTE COLLECTION

1. PURPOSE

This Standard Operating Procedure (SOP) intends to describe the procedure for collecting oocytes in *Xenopus laevis*.

2. RESPONSIBILITY

Principal investigator (PI) and their research staff.

3. MATERIALS

- 3.1. Powder-free, non-textured gloves
 - 3.2. Anesthetics
 - 3.3. 0.9 % Saline
 - 3.4. Sterile surgical instruments
 - 3.5. Sterile cotton-tipped swabs
 - 3.6. Suture material 4-0 synthetic monofilament absorbable with a reverse cutting needle (e.g., Assucryl (R))
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4. PROCEDURE

- 4.1. Moisten gloves with tank water before handling frogs.
- 4.2. Alternate between the right and left ovaries for each oocyte collection procedure.
- 4.3. Allow for a 6-month recovery period between surgical procedures.
- 4.4. Frogs must not be fed on the day of surgery to avoid emesis while anesthetized.
- 4.5. All post-surgical animals must be appropriately identified.
- 4.6. Surgical Principles/Aseptic technique:
 - 4.6.1. Designate a sterile area on the working surface for the sterile material (instruments, suture material, gauze, etc.).
 - 4.6.2. Begin surgery with clean and sterile surgical instruments and handle them aseptically. Sterilize instruments by autoclaving or by using a glass bead sterilizer.
 - 4.6.3. Avoid liquid cold sterilization solutions, as these chemicals should not encounter the frog's permeable skin.
 - 4.6.4. Disinfect the instruments between each animal by dipping them in a hot glass bead sterilizer for approximately 15 seconds after removing any blood and debris (let cool completely).

4.7. Surgical Procedure:

- 4.7.1 Anesthetize the animal per Fish and Aquatic Amphibian anesthesia SOP202. Hypothermia is not recommended as an adjunct to anesthesia as it will prolong recovery.
- 4.7.2 Identify the frog by color mark, assess the general appearance, and weigh her. Log your findings in the BGU preclinical management system or a logbook in the frog room.
- 4.7.3 Before surgery, verify the anesthesia depth by losing the animal's pedal withdrawal (toe pinch) reflex.
- 4.7.4 Keep the skin moistened during surgery.
- 4.7.5 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 infection.
- 4.7.6 Inject local analgesia (Lidocaine < 2mg/kg SC) at the area of the intended surgical incision.
- 4.7.7 Make a small incision (1-1.5cm, using number 15 or 11 scalpel blade) on the abdomen above the groin, lateral to the midline.
- 4.7.8 Use blunt scissors to dissect through the fascia and muscle to visualize the oocytes.
- 4.7.9 Oocyte strands are then gently externalized and cut.
- 4.7.10 One stitch using an absorbable suture may be taken in the ovary to control bleeding.
- 4.7.11 After ensuring no hemorrhage from the surgical site, the abdominal muscles, and skin layers are sutured in two separate layers. Absorbable monofilament sutures such as Assucryl (R) should be used for skin closure (3-0 or 4-0 suture is generally adequate).

4.8 Post-operative Care:

- 4.8.1 Recovery from the anesthesia can take up to 30 min. Place the animal in an anesthetic-free tank water bath (shallow water level) and rinse it with fresh, well-oxygenated water to hasten the recovery process.
- 4.8.2 Separate the frog into a recovery tank. Check the frog for general appearance, muscle tone, and mobility every 5 minutes.
- 4.8.3 Frogs should be **monitored daily for three days and then 2-3 times a week** for the next two weeks for appetite and any complications such as dehiscence (herniation) or surgical site infection.
- 4.8.4 *XENOPUS* Procedure Log must be filled out (SOP501b) and kept in the frog room.

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Approved by the BGU Animal Policy and Welfare Oversight Committee