

## FLSC Standard Operating Procedure for Surgical Oocyte Removal from the *Xenopus*

### Equipment:

4-0 Absorbable suture with taper needle	Povidine 3x3 gauze sponges
System Water	Paper Towels
Ceramic Crock- small	200 ml Flask
Litmus Paper	50 ml beaker
Spatula	Plastic aquarium with lid
Sterile Surgical Pack	Scale
Bench-cote®	
0.2 g MS-222 powder - Ethyl 3-aminobenzoate methanesulfonic acid salt (1g/L concentration)	
0.2 – 0.8 g Baking Soda – Sodium bicarbonate (1-4 g/L )	
PPE: Nitrile gloves, safety glasses, and lab coat	

### Surgical Pack is composed of the following instruments:

1 Watchmaker's forceps or Dressing forceps	1 Rat tooth forceps or Brown Adson forceps
1 Olsen-Hager Needle Holding forceps	2 pair Iris scissors

### Preparation:

- Mix the immersion anesthetic solution.
  - Measure MS-222, using the spatula, into the 50 ml beaker on the electronic scale being careful to avoid skin contact.
  - Add a small amount of cold tap water to the MS-222 powder and dissolve using the spatula.
  - Add cold tap water to the dissolved MS-222 bringing the total volume to 200 ml.
  - Mix well and pour into the ceramic crock.
  - Buffer to a pH between 7.0 -8.0 with 0.2g increments of sodium bicarbonate powder mixing until dissolved.
  - Check the solution pH by placing a drop onto litmus paper and determine the pH.
  - Continue to add 0.2 g of sodium bicarbonate powder to the anesthetic solution and checking the pH until it is within the 7.0-8.0 range.
- The surgical table is cleaned with disinfectant spray and rinsed with alcohol.
- The surgical instruments and suture are opened using the opened surgery pack as the sterile field.
- Place Bench-cote® or a comparable product on the table with the water repellent side down. Place a moistened paper towel on top the Bench-cote®. The surgery will be performed with the *Xenopus* on the moist paper towel to prevent the *Xenopus* from sticking to the paper during surgery.

### Anesthesia:

- The *Xenopus* is placed in the crock and the aquarium lid is placed over the opening to contain the animal in the anesthetic solution.
- Due to the variable induction times with each individual *Xenopus*, it is necessary to monitor the anesthesia depth by stimulating the frog at regular intervals.
- The criteria for assessing anesthetic depth at a surgical plane of anesthesia includes:
  - Loss of leg tone – the animal does not struggle or withdraw the leg when the back leg is extended.
  - Loss of toe pinch response – the animal does not respond to a forceps pinch to the toes on a hind leg.
  - If there is movement or increase in respirations, the animal is not at a surgical plane of anesthesia.
- Anesthesia will last for about 15 – 20 minutes.

**Surgery:**

1. The most cranial incision permitted is ~ ¼ inch or one finger width below the termination of sternum, thereby reducing the potential for damaging the liver or heart when making an incision or removing oocytes.
2. Incisions must be carefully spaced, alternating sides. If necessary, when working with individual frogs having shorter body lengths, additional incisions may be placed on the midline.
3. The *Xenopus* is placed in dorsal recumbency on the moist paper towel.
4. Swab the abdomen with a Povidine gauze sponge.
5. Grasp the skin in the lower left or right abdominal area with the thumb forceps about 1 cm from the midline. The skin fold should be parallel to the midline.
6. Make a small cut perpendicular to the midline with the iris scissors. This cut should not bleed and should be no longer than 5-7 mm.
7. Make a second incision through the muscle layer no larger than the skin incision.
8. Reach into the incision using the forceps and gently grasp the egg filled ovarian lobe. Point the forceps caudally to avoid puncturing the liver. Withdraw the eggs through the incision with a steady traction.
9. Never grasp across the ovarian lobe as you withdraw eggs from the coelomic cavity as that will crush and damage the eggs. Instead grasp the edge of the membrane and rock the lobe left and right to extract the lobe and the eggs.
10. Once the desired quantity of eggs has been withdrawn cut through the lobe and place the eggs into a beaker with solution provided by the investigator.
11. Close the muscle layer with 4-0 absorbable suture on a taper needle using one to two simple interrupted sutures.
12. Close the skin with either a single mattress suture or one to two simple interrupted sutures.

**Recovery:**

1. A benefit of MS-222 anesthesia is that it has analgesic properties and removes the necessity of administration of post surgical analgesia in most cases.
2. Create a floating pillow comprised of 2 – 3 crumpled paper towels covered with several additional paper towels. This is submerged in the water trapping air in the inner towels.
3. Place the animal on the paper towels in the clear aquarium containing 3 to 4 inches of system water. This water contains sodium chloride, is warm, and decreases recovery time.
4. Monitor the animal until voluntary movement is observed.
5. Return the animal to the original tank once she is moving.
6. Record the surgery in the Survival Surgery Log and on the cage card.
7. Check the skin suture for 2 days post-operatively.

**Terminal Surgery:**

1. The 8<sup>th</sup> surgery is a terminal procedure.
2. Anesthetize and prepare the animal for surgery as listed above.
3. The body cavity will be opened with a midline incision and all eggs harvested. On occasion the liver may be removed as well.
4. Sever the heart and follow with decapitation or pithing to ensure death.

**Precautions:**

1. A buffered anesthetic is prepared fresh for each surgery. Used anesthetic solution can be saved as euthanasia solution and stored in the refrigerator according to the SOP for Storage of Immersion Euthanasia Solutions.
2. Nitrile gloves must be worn when handling any immersion anesthetic.
3. Once the muscle layer has been cut, the animal cannot be returned to the anesthetic solution.
4. Oocyte removal surgeries on a single animal are allowed up to a maximum of 7 survival surgeries with a final 8<sup>th</sup> terminal surgery.

5. Surgeries may be limited to fewer than 8 due to the size or body condition of the animal. In those cases the investigator will be informed.
6. Avoid trapping any eggs under the skin. If eggs are trapped between the muscle and skin, the *Xenopus* may have a fatal reaction or develop an abscess.
7. *Xenopus* must break the surface of the water to breathe so it is imperative that the nose of the animal be elevated above the water line until it has recovered sufficiently to swim and reach the surface.
8. The portion of the *Xenopus* exposed to air during recovery must be kept moist to avoid injury to the skin.
9. Adequate recovery time must be allowed between surgeries. Frogs will be rotated so that the interval between surgeries is maximized. Recovery times should not be less than 2 weeks between surgeries.
10. Incisions for oocyte removal will alternate between right and left sides.