

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

Equipment and Supplies:

4-0 Absorbable suture with taper needle	Povidine 3x3 gauze sponges
System Water	Paper Towels
Ceramic Crock- small	200 ml Flask
Nitrile Gloves	50 ml beaker
Spatula	Plastic aquarium with lid
Sterile Surgical Pack	Scale
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)	
Litmus paper	

Surgical Pack is composed of the following instruments:

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

Preparation:

A buffered anesthetic is prepared fresh for each surgery. Nitrile gloves must be worn. The MS-222 powder is stored in the freezer. Measure the 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 water to the MS-222 powder and dissolve using the spatula. Mix anesthetic solution by adding the dissolved MS-222 to the flask and bringing the total volume to 200 ml with cold tap water. Mix well and pour into the ceramic crock. Measure 0.2 g baking soda. Add this to the MS-222 solution mixing until dissolved. Place a drop onto litmus paper and determine if the pH is between 7.0 and 8.0. Continue to add 0.2 g of baking soda to the anesthetic solution and checking the pH until it is within the 7.0-8.0 range. The table is cleaned. The surgical instruments rest on the sterile peel pack once it is opened. The surgery will be performed with the *Xenopus* on a moist paper towel to prevent the *Xenopus* from sticking during the surgery.

Anesthesia:

The *Xenopus* is placed in the crock and the aquarium lid used to keep the *Xenopus* in the crock. The induction time varies with each individual *Xenopus*. It is necessary to monitor the anesthesia depth by stimulating the frog at regular intervals. The *Xenopus* loses leg tone and does not struggle when the back leg is extended followed by loss of the toe pinch response. Use the forceps to pinch on of the toes on a hind leg. If there is no movement or increase in respirations, a surgical plane of anesthesia has been reached.

Surgery:

Oocyte removal surgeries on a single animal are up to a maximum of 7 survival surgeries with a final 8th terminal surgery. The most cranial incision permitted is ~ ¼ inch or one pencil width below the termination of sternum, thereby reducing the potential for damaging the liver or heart when making an incision or removing oocytes. For this reason, subsequent incisions must be carefully spaced, alternating sides. If necessary, when working with individual frogs having shorter body lengths, incisions may be placed on the midline. In some instances, surgeries may be limited to fewer than 8. In those cases the investigator will be informed. The *Xenopus* is placed in dorsal recumbency on the moist paper towel. The surgical instruments and suture are opened. Swab the abdomen with the Povidine gauze sponge. Using forceps grasp the skin in the lower left or right abdomen about 1 cm from the midline. The skin fold should be parallel to the midline. With the iris scissors make a small cut perpendicular to the midline. This cut should not bleed and should be no longer than 5-7 mm. A second incision is made through the muscle layer of the abdomen no larger than the first. The eggs can be extracted through this incision. Using the forceps gently grasp the egg filled ovarian lobe and with steady traction pull the eggs through the incision. Using the iris scissors, cut off the desired quantity of eggs. The incision is closed with absorbable suture on a taper needle. One to two simple interrupted sutures are placed in

the muscle layer and a single suture placed in the skin. 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.

Recovery:

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. The *Xenopus* is placed in the clear aquarium with 3 to 4 inches of system water. This water contains sodium chloride, is warm, and decreases recovery time. Because the *Xenopus* must break the surface of the water to breath, several paper towels are crumpled and placed in the water to support the head. The *Xenopus* must be monitored until voluntary movement is observed. The portion of the *Xenopus* exposed to the air must be kept moist to avoid injury to the skin. Once the *Xenopus* is moving freely she can be replaced in her original tank. The surgery is recorded on the cage card and in the Survival Surgery Log. The skin suture is checked for 2 days post-operatively. Adequate recovery time must be allowed between surgeries. Incisions for oocyte removal will alternate between right and left ovaries. Frogs will be rotated so that the interval between surgeries is maximized. Recovery times should not be less than 2 weeks between surgeries.

Terminal Surgery:

The 8th surgery is a terminal procedure. The frog will be anesthetized and prepared for surgery as listed above. The body cavity will be opened with a midline incision and all eggs harvested. On occasion the liver may be removed as well. The heart is severed followed by decapitation and pithing to ensure death.