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## **SURGICAL (SURVIVAL) OOCYTE COLLECTION FROM XENOUS LAEVIS**

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### **PURPOSE:**

- This document describes the procedure for aseptic surgical (survival) collection of oocytes from *Xenopus laevis* following the Canadian Council on Animal Care (CCAC) current guidelines on amphibians.

### **RESPONSIBILITIES:**

- All those trained persons listed on an approved Animal Care Committee (ACC) Animal Use Protocol (AUP) who are performing this procedure

### **MATERIALS:**

- Dedicated surgical area
- Appropriate disinfectant for surgical work surface (i.e.: 70% Alcohol or Virkon)
- Clean, non-bleached paper towels, non-abrasive towels or bench top cover
- Sterilized (autoclaved) surgical instruments (i.e. needle drivers, iris scissors, rat-toothed forceps, non-toothed forceps).
- Hot bead sterilizer if multiple surgeries on same day are to be performed
- Appropriate suture material (5-0 Sterile monofilament absorbable and non-absorbable)
- Sterile cotton-tipped applicators
- Sterile 0.9% sodium chloride
- Dechlorinated water
- Transport tank with lid
- Thermometer
- Clean lab coat
- Hand soap
- Clean, non-textured, powder free, nitrile gloves
- Appropriate, buffered anesthetic (such as MS-222)
- Appropriate analgesics (see below)
- Recovery tank with lid and clean, dechlorinated water the same temperature as housing tank
- Tank card
- Procedural/surgical records
- Post-operative monitoring sheets

## PROCEDURE:

### Surgical Oocyte Removal

#### 1. Candidate selection and preparation

- a. Animals must be in good health, there must be sufficient time (as approved in the AUP) between collections to allow the animal to recover. In general, a recovery period of 3 to 6 months is appropriate.
- b. If the animal has experienced a previous surgical oocyte removal, plan to perform the surgery on the contralateral side.
- c. Amphibians may be fasted for a maximum of 24 hours before surgery

#### 2. Preparation of the surgical area

- a. The surgical area should be a dedicated space that is easily cleaned and disinfected, is uncluttered and away from the flow of traffic to minimize contamination from other laboratory activities.
- b. The work surface (table or counter top) must be non-porous and must be thoroughly cleaned with an approved disinfectant before and after each surgical procedure.
  - i. Common disinfectants include: 70% alcohol or Virkon. Allow the surface to dry completely before the frogs come in contact with it. Virkon residue can be removed with 70% alcohol if needed.
- c. A clean work surface such as a clean, cloth drape, non-bleached paper towel or disposable benchtop cover should be used for each animal and should be cleaned or disposed of between animals. These should be moistened with tank water to prevent the skin from drying out or sticking.

#### 2. Preparation of the surgical instruments

- a. All surgical instruments must be sterilized prior to use. Acceptable sterilization methods include: autoclaving (steam or dry heat) or ethylene oxide. The use of chemical sterilants should be avoided as these toxic chemicals can be inadvertently introduced into the surgical site or contact the skin. **Alcohol is NOT a sterilant.**
- b. A sterile field (sterile cloth, sterile paper or sterile drape) must be used to lay the sterile instruments on with care taken not to contaminate the sterile tips of the instruments when placing and replacing the instruments on the sterile field.
- c. It is recommended that a separate set of autoclaved instruments is used for each frog. If multiple frogs are having surgery in one day, a hot bead sterilizer can be used to re-sterilize the tips of the instruments after the first surgery. The instruments must be cleaned of any bodily fluids or debris with sterile water or saline prior to being placed into the hot bead sterilizer. Sterilization of the tips occurs within 15 seconds. The instruments **MUST** be allowed to cool to room temperature prior to use and care must be taken that the sterile tips are placed on a sterile field. The hot bead sterilizer must only be used on instruments between 6 frogs (or a maximum of 4 hours) before being fully re-sterilized.

#### 3. Preparation of surgeon

- a. The surgeon must wash their hands before and after each surgical procedure

(regular hand soap is fine).

- b. The surgeon must wear a clean lab coat.
- c. Though sterile gloves are not required, the gloves must be changed between surgeries and care must be taken to not touch the sterile instrument tips, tissue or sterile suture with the non-sterile gloves.
  - i. Non-textured, powder-free Nitrile or vinyl gloves are recommended.

#### **4. Preparation of the frog**

- a. **Choose a healthy female frog who is normally active and confirm that the time since her last surgery is at least 3 to 6 months as approved in the AUP.**
- b. Moisten gloves with tank water when handling frogs.
- c. Transport frog in water from their home tank to and from surgical area.
- d. Anesthetize frog as described below.
- e. Lids should be used to prevent frogs from jumping out of any container.

#### **5. Preparation of MS-222 (Tricaine methane sulfonate)**

- a. MS-222 (typically 300-500 mg/L) must be made fresh the day of the planned surgery or at least within 24 hours of use. Ensure solutions are protected from light, discard discoloured solutions.
- b. Prepare MS-222 in a fume hood while wearing gloves and a lab coat.
- c. The MS-222 must be buffered to a pH of 7.0-7.4 using sodium Bicarbonate (NaHCO<sub>3</sub>).
  - i. Neutrality must be confirmed with a pH meter or pH paper.
  - ii. Unbuffered MS-222 is very acidic and can cause skin damage to the frogs. Buffered MS-222 is also more bioavailable and will result in smoother inductions since it is better absorbed.

#### **6. Anesthetizing the frog**

- a. Check that the temperature of the MS-222 is the same as the housing tank's water.
- b. Submerge the body of the frog, keeping its nostrils above water in the MS-222.
  - i. The frog must not be left unattended and must be observed at all times during anesthesia, surgery and during recovery until it is able to swim normally.
  - ii. Frogs will drown if their nostrils are submerged as they become anesthetized.
  - iii. It may take 5-20 minutes for frogs to become anesthetized.

#### **7. Administration of analgesia**

- a. Analgesia is required for recovery surgery and should be administered before the surgery and when the frog is anesthetized.
  - i. The following agents have been successfully used in *X. laevis*:
    - Bupivacaine or lidocaine diluted to 0.25%: Either may be swabbed onto the skin or may be infiltrated at the incision site and allowed time to absorb prior to surgery (5 minutes). Do not exceed 5 mg/kg total dose either topical or intra-incisional. Lidocaine has a quicker onset of action but wears off quicker (60 minutes) while bupivacaine has a longer onset of action (30 minutes) but can provide pain relief for 3-4 hours.
    - In addition or alternatively, the following analgesics are recommended\*\*:
      - Banamine (flunixin meglumine) 1 mg/kg SC
      - Meloxicam 0.1 mg/kg SC

- Buprenorphine 75 mg/kg SQ
- Fentanyl 0.5-1.0 mg/kg SQ
- Meperidine 100 mg/kg SQ
- Dexmedetomidine 40 mg/kg SQ
- Xylazine hydrochloride 10 mg/kg injected intracoelmically

\*\*In certain applications, all of the drugs above may impact certain receptors in the oocytes so this should be determined before a large scale collection of oocytes is undertaken using these drugs.

The addition of analgesics can cause the depth of anesthetic achieved by MS-222 to deepen so close observation of the frog will be necessary and the amount of anesthetic adjusted as required.

## 8. Determination of depth of anesthesia

- As frogs become anesthetized, they lose muscle tone and become inactive.
- A surgical depth of anesthesia will usually last between 10-20 minutes.
- A surgical depth of anesthesia is reached when frog displays:
  - A regular and relaxed respiratory rate.
  - Absence of withdrawal reflex when hind feet pinched (main reflex indicating proper surgical depth).
  - Absence of muscle tone when hind leg extended.
  - No response to external stimuli.
  - Absence of gular (throat) movements
- The depth of anesthesia must be checked every 5-10 minutes along with physiological parameters (breathing, muscle tone).
- If the frog is not fully anesthetized or the procedure lasts longer than the anesthesia, a weaker dilution (typically 50% of the initial anesthetic dose) of the buffered MS-222 can be dripped directly onto the skin away from the surgical site.

## 9. Surgery

- Place the appropriately anesthetized frog in dorsal recumbency (lying flat on its back) on moistened non-abrasive towels or benchcoat.
- Surgical scrub solutions must NOT be used since the skin of amphibians is very permeable and the solutions will be absorbed systemically. Instead, rinse the skin with sterile saline solution. Do not rub the skin.
- Paper or cloth sterile drapes may disrupt the natural protective mucous layer of the skin. Sterile saline-moistened plastic surgical drapes may be used, leaving a small window where the skin incision will be made, uncovered.
- The skin of the frog needs to be kept moist with sterile physiological (0.9%) saline throughout the procedure.
- Using sterile 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 sterile 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 and in the same orientation as the first incision. Tent the muscle layer to make the incision to avoid puncturing organs or lymph hearts. The eggs can be extracted through this incision.
- Using the forceps, gently grasp the egg filled ovarian lobe and with steady traction exteriorize the egg mass onto the sterile plastic drape. The ovarian mass should not come into contact with the frog's skin.
- Using the iris scissors, cut off the desired quantity of eggs.

- i. There should be no bleeding associated with the excision.
  - ii. A single, absorbable monofilament suture can be used to suture the remaining ovarian tissue. Care must be taken not to contaminate the suture by allowing it to touch the skin or non-sterile surfaces.
  - iii. The remainder of the egg mass should be carefully replaced into the coelomic cavity
- j. The muscle incision is closed with 5-0 monofilament, absorbable suture on a taper needle. One to two simple interrupted sutures are placed in the muscle layer.
- k. The skin incision is closed with 5-0 non-absorbable, monofilament suture on a taper needle. One or two simple interrupted sutures are placed in the skin.
- l. 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.

## 10. Recovery

- a. The frog should be rinsed off in clean, chlorine-free water that is the same temperature as the tank water to remove traces of the MS-222 and speed recovery.
- b. The frog is then placed into a separate small container with a slant so that the lower body can be submerged in water with the head and nostrils elevated. Cover the exposed skin with a wet drape.
- c. Ensure that you use chlorine-free water which is at the same temperature as the home tank water
- d. The frog must be observed at all times during recovery as they can drown if their nostrils slip below the water surface.
- e. Recovery from the anesthesia can take up to one hour.
- f. Post-recovery monitoring should take place according to the AUP.
- g. Once the frog is moving normally and able to swim and dive, she can be brought back to her regular housing room and placed into a recovery tank with the normal amount of water. Typically frogs recovering from surgery are housed separately from their original tank mates until they are observed to be eating and recovering normally at which point they can be re-introduced. Ensure the tank has proper tank card indicating protocol number, PI, numbers of frogs present, and date and type of procedure that was performed.
- h. Document the side the oocytes were removed from, all drugs, doses and routes of administration for the anesthetics and analgesics, any complications and post-operative observations (may be recorded on the cage card, surgical monitoring record or procedure log).
- i. The frog must be checked daily and written records kept (including weekends and holidays) observing for signs of:
  - j. Eating normally
  - ii. Swimming normally
  - iii. Incisional healing or complications (swelling, discharge, etc)
  - iv. Skin lesions or loss of skin
  - v. Swollen or red legs
- i. The full healing of the incision and suture removal must be recorded.
- j. All sick or found dead frogs must be reported to the UBC Clinical Veterinarians.

## REFERENCES

- UBC Animal Care Committee Policy #17 (Policy on Monitoring of Animals used for Research, Teaching and Testing).  
<https://animalcare.ubc.ca/sites/default/files/documents/Policy%2017%20Policy%20on%20Moni>

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- Green SL. 2009. The laboratory Xenopus sp. Boca Raton (FL): CRC Press.