

**Stony Brook University
Institutional Animal Care and Use Committee (IACUC)**

SURGICAL COLLECTION OF OOCYTES FROM FROGS

A. Anesthesia

- 1) Anesthesia is required for surgical removal of oocytes.
- 2) Frogs are anesthetized by immersion in a Tricaine (Tricaine methanesulphonate) solution. Tricaine should be adjusted to neutral pH.
 - a) tadpoles/larvae – 0.05% solution (0.5 g/L)
 - b) adults frogs/urodeles - 0.1-0.2% solution (1-2g/L)
 - c) toads (Bufo sp.) – 0.3% solution (3g/L)
- 3) Anesthesia is maintained by keeping the frog partially immersed in a small amount of Tricaine solution during the surgical procedure.
- 4) Ice should not be added to the anesthetic solution.
- 5) The use of any other anesthetic agents must be justified in the IACUC application.

B. Anesthesia Monitoring

- 1) A surgical plane of anesthesia should occur within 20 minutes of immersion into the solution. Erythema of the ventrum or other light skinned areas of the body is the first sign of anesthesia induction.
- 2) A light plane of anesthesia is characterized by a loss of righting reflex and corneal reflex. The withdrawal reflex, spontaneous movement and cardiac impulse (visible heartbeat) are retained.
- 3) A deep plane of anesthesia is the stage when only the cardiac impulse is present. The withdrawal reflex is the last to go.
- 4) While inducing, and during the procedure, the following parameters must be monitored at a minimum of 5 minute intervals:
 - Respiratory rate
 - Righting and corneal reflex
 - Response to noxious stimulus
 - Spontaneous movement
 - Cardiac impulse (visible beating)

C. Anesthesia Recovery Monitoring

- 1) Rinsing the body in distilled, well-oxygenated water will reverse the anesthetic effects.
- 2) During recovery from anesthesia, the following clinical parameters must be monitored at a minimum of 5 minute intervals until the animal is ambulatory.
 - Respiratory rate
 - Spontaneous movement
 - Muscle tone
- 3) Recovery should take approximately 15 minutes.

D. Surgical Procedure

- 1) After the animal attains a surgical plane of anesthesia, a gauze pad, soaked in betadine solution, should be applied to the abdomen. Do not rub the skin with betadine.
- 2) Surgery should be performed using aseptic techniques:
 - a) The surgeon should wear mask, sterile gloves, a lab coat and a head cover.
 - b) All instruments must be pre-sterilized by acceptable methods, including steam sterilization, Cidex™ cold sterilization or by the use of a glass bead sterilizer. Instruments must be re-sterilized between animals. When performing surgery on more than one animal, effective sterilization can only be practically achieved by use of a glass bead sterilizer or by pre-sterilization of multiple sets of instruments. Cidex™ cold sterilization requires 10 hours of contact time to be effective. Dipping instruments in 70% alcohol between surgeries does not achieve sterility (>30 hrs of contact time required) and is not an acceptable method.
- 3) An incision is made in the abdomen, parallel and lateral to the midline.
- 4) A section of ovary containing several dozen oocytes is removed. Avoid exteriorizing the ovary outside of the abdominal cavity.
- 5) Place one absorbable suture in the ovary to control bleeding.
- 6) Suture the abdominal muscle and skin.
- 7) The surgical procedure takes approximately 15 minutes.

E. Analgesia

No analgesia is required for this procedure.

F. Surgical Parameters

- 1) If frogs are to have multiple survival surgeries performed, tissue should be removed from the other ovary and surgery should not be performed until the initial incision has completely healed (approximately 2-3 months).
- 2) The number of multiple survival surgeries performed on any one animal should be determined by the animals clinical appearance (i.e. incisions that have healed properly, maintenance of weight, good activity level) and quality of oocytes produced.
- 3) No more than 5 multiple survival surgeries may be performed on any one animal. Additional multiple survival surgeries must be justified by the investigator and approved by the IACUC.

G. Adverse Effects

Potential adverse effects from these surgical procedures include:

- anesthetic related respiratory distress
- infection of the surgical site
- non-healing of the surgical site
- peritonitis

H. Clinical Monitoring and Management

- 1) Frogs must be segregated post-operatively into a small recovery cage for a minimum of 2 weeks to facilitate monitoring.
- 2) Frogs must be monitored at least twice weekly after each surgical procedure.
- 3) Any frog with an incision that is not healing properly (open, infected, red) or animals that appear to be losing weight should be reported to the DLAR Veterinary staff so that appropriate medical treatment can be initiated.
- 4) If animals have acute adverse reactions to the anesthetic agents (respiratory distress and/or lack of recovery), they must be euthanized immediately.

I. Early Endpoints

- 1) Animals should be euthanized if the surgical incision dehisces or if treatment of an infected incision site is unsuccessful.
- 2) After an animal has had 5 multiple survival surgeries performed (as evidenced by the number of surgical scars on the abdomen), the animal should be euthanized.