

Multiple Survival Surgery (Xenopus)

I. Overview

Xenopus laevis (the African clawed frog) oocytes are surgically harvested and used for a variety of experiments in basic science research. Oocytes are collected by anesthetizing the frog, and making a small coelomic incision. The quality of the oocytes varies a great deal from frog to frog. Thus, the reproducibility of critical experiments can be enhanced if the oocytes are collected from a consistently good oocyte producer. For this reason, multiple major survival surgeries are acceptable in *Xenopus* for the collection of oocytes, provided the following guidelines are followed.

II. Guidelines:

- The **maximum** number of surgeries allowed per animal is six, three on alternating sides of the coelomic cavity, with the sixth surgery being a terminal procedure.
- The interval between procedures should be no less than one month.
- A written plan of action must be included in the protocol as to the method used to identify animals to ensure adequate time has lapsed between surgeries. Examples are tattooing and tank rotation.

a. Aseptic Surgical Technique:

- Aseptic technique must be used to reduce microbial contamination to its lowest possible practical level. In keeping with this mandate in consideration of the species, the following is deemed acceptable aseptic technique:
 - Surgical instruments must be sterilized by either heat, gas, or chemical sterilants followed by a rinse in sterile water or saline. Alcohol is not a sterilant.
 - As the frog skin should not be cleansed, sterile gloves are not required.
 - The container in which the animal is placed should be clean.
 - The anesthetic solution used on each animal should be fresh or sterile filtered as this will also serve to minimize possible contamination.

b. Anesthetic:

- Use of general anesthesia (for example, MS-222) is required. Chilling frogs on ice is not considered an acceptable method of anesthesia. During anesthesia and recovery, care must be taken to ensure the nares are not submerged in liquid.

c. Postoperative Care:

- Postoperative care for each animal should include at a minimum singly housing the animals and monitoring the wound healing within 24-48 hours

post surgery. If the animal is being maintained in a translucent rodent cage, the incision can be checked by looking through the bottom.

- Skin sutures and wound clips, if nonabsorbable, must be removed 2-3 weeks after surgery.
- Records must be maintained to ensure that proper animal care is occurring. Record keeping should include the number of surgeries per animal and a description of postoperative care.

d. **Euthanasia:**

- Acceptable methods of euthanasia include anesthesia overdose or anesthesia followed by decapitation or pithing.