

Policies and Procedures

SECTION: IACUC	NUMBER: 7.02			
CHAPTER: Miscellaneous Experimental Animal Use Policies	ISSUED: 6/2007	REV. A: 4/7/2009	REV. B: 11/2012	REV. C: 08/2016
POLICY: Harvesting of <i>Xenopus laevis</i> eggs and oocytes	REV. D:	REV. E:	PAGE 1 OF 2	

Purpose

The purpose of this policy is to provide clear guidelines for harvesting egg and oocytes in *Xenopus laevis*. This procedure is approved by the Creighton University Institutional Animal Care and Use Committee (IACUC). All investigators will follow this policy unless scientific justification is provided and approved by the IACUC.

Application

Amphibian oocytes are used for studies in molecular biology, embryology and biochemistry. Stage I-VI oocytes are often obtained by surgical laparotomy. Multiple surgeries on a single animal may be justified for scientific reason, considering the lack of complications when performed by competent personnel. Unfertilized eggs are collected by gently squeezing eggs from females which have been injected with human chorionic gonadotropin (HCG) - 500 IU injected into the dorsal lymph sac. Females should begin laying eggs 12 hours after the HCG injection. When properly performed by technically proficient research personnel, female *Xenopus* are not harmed by the egg stripping procedure and can be used again after a recovery period of 3-6 months. The total number of laparotomies should be limited and will depend on the health of the animal and quality of the oocytes as well as the life span of the animal and the duration of egg production. Up to a maximum of five recovery surgeries (with the final 6th terminal surgery) per animal are acceptable.

1. Surgeries should be performed by trained personnel using appropriate anesthesia such as tricaine methane-sulfonate (MS-222). MS-222 solution should be buffered to a neutral pH of 7.0. Cooling is not acceptable for use as an anesthetic. The current AVMA Guidelines for euthanasia indicates that "there is no evidence that whole body cooling reduces pain or is clinically efficacious."
2. Surgeries must be performed using aseptic technique appropriate for amphibians. This will include the use of sterilized instruments and gloves. Instruments should be sterilized by autoclaving or using a glass-bead sterilizer. The use of cold sterilants should be avoided so that these potentially toxic chemicals are not inadvertently introduced into the surgical site or onto permeable amphibian skin. The use of surgical drapes and preparation of the surgical site remains controversial for aquatic species.
3. The use of a sterile drape and preparation of the surgical site with dilute povidone iodine or chlorhexidine solution has been recommended (DP O'Rourke and TW Schultz, *Biology and Diseases of Amphibians, Laboratory Animal Medicine, 2nd Edition*, Academic Press, 2002). However, another source notes: "Fish skin is easily damaged by alcohols and surgical scrub solutions, and the exuberant response of the mucus-producing glands to that damage far exceeds any potential benefits derived from using

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them.” (MK Stoskopf, Surgery, Fish Medicine, W.B. Saunders Company, 1993). The use of these chemical agents may disrupt the normal skin flora of the patient and the constant mucous production of *Xenopus* skin makes any sterilization effort transient. When chemical surgical preps are used, they should be limited to the immediate area around the incision site and should only be solutions, not scrubs containing soaps or detergents.

4. Similarly, arguments have been presented regarding the use of surgical drapes. Drapes may be useful to keep mucus from getting on instruments and suture material, and can be moistened to keep skin from drying during surgery. However, amphibian skin can be easily damaged and paper drapes that become wet pose no barrier to bacteria. NIH veterinarians report that the incidence of clinical complications following surgical oocyte harvesting is rare. The decisions regarding the use of surgical drapes and performing single surgical site skin preps are left to the discretion of the Principal Investigator in consultation with the attending veterinarian and veterinary staff and with approval by the IACUC.
5. Both tissue layers must be closed separately using a monofilament absorbable suture material (3-0 to 4-0) via a simple interrupted suture pattern.
6. Single housing for 2-3 days after surgery should be provided as part of the post-surgical care of laparotomized animals. Frogs must be monitored daily for at least one week post-operatively. Appetite as well as for any complications such as dehiscence or infection should be documented. Care will be provided as approved in the animal use protocol. Adequate recovery time should be allowed between laparotomies. The investigator can alternate oocyte collection between left and right ovaries and consider rotation of frogs so that the interval between surgeries in any individual is maximized. Ideally frogs should be rested at least two months between laparotomies; shorter resting periods may be appropriate if only small amounts of tissue are harvested. Recovery time of less than one month should have prior approval of the IACUC.
7. Although use of post-operative analgesics is not well defined following oocyte harvest in amphibians, administration of flunixin meglumine (25 mg/kg) should be injected into the dorsal lymph sac for animals exhibiting a loss of appetite. Consultation with the Attending Veterinarian is required for guidance.
8. Investigators should consider methods to individually identify animals which receive surgery in order to track how many surgeries are performed on a given animal. Identification may include but are not limited to individual housing, color-coded beads, sutured to the animal’s skin, subcutaneous dyes or a photography log of the unique patterns on each animal’s dorsum