



Institutional Animal Care and Use Committee Guidelines and Policies

February 20, 2008

GUIDELINES FOR OOCYTE HARVESTING FROM FROGS

Xenopus oocytes are used in biochemistry and molecular biology for the expression of proteins and electrophysiological recordings. Stage I-VI oocytes are usually harvested by surgical laparotomy. Because an individual frog may serve as a source of thousands of oocytes over their lifetime, multiple survival surgeries are frequently performed. *The Guide for the Care and Use of Laboratory Animals* discourages multiple survival surgeries and requires adequate justification and IACUC approval before the procedures are performed. IACUC recognizes that multiple oocyte harvests from an individual frog reduces the total number of animals used and enhances the quality of the research by allowing multiple collections from animals that produce high quality oocytes. However, the reduction in the number of animals used must always be balanced with the need to minimize the pain and distress experienced by individual frogs. To that extent, the IACUC has developed the following guidelines regarding the harvest of oocytes by surgical laparotomy:

1. Surgeries should be performed by trained personnel using appropriate anesthesia such as tricaine methane-sulfonate (MS-222). Surgeries should be done as aseptically as practical including 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.
2. Based on common practices in the field of biomedical research, the maximum number of surgeries allowed per individual animal is limited to five survival surgeries and one terminal procedure (total of six surgeries). The total number of surgeries performed and interval between surgeries should take into account the physiological condition of the frog and the quality of oocytes produced.
3. Adequate recovery time should be allowed between laparotomies. The interval between survival procedures should be no less than one month to allow for recovery and healing. Sutures should be removed after 4 weeks if they have not been sloughed by this time. Each subsequent surgery should be performed on the contralateral side such that oocyte collection alternates between left and right ovaries. Evidence of surgery-related stress such as poor body condition, poor oocyte quantity or quality, and/or clinical disease will require an extended rest period based on recommendations from the veterinary staff. *Recovery times of less than one month must be justified on scientific grounds and approved by the IACUC.*
4. The investigator should rotate frogs so that the interval between egg collections is maximized. Frogs should be individually identified or placed into a formal rotation system that prevents animals from being reused more frequently. Cage cards must have surgery dates indicated; beads sewn onto the skin can be used to identify individual frogs that are group housed.



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5. Single housing or small group housing for several days after surgery should be considered as part of the post surgical care of laparotomized animals. Frogs should be monitored daily during this period to ensure they are eating properly, as well as to detect any complications such as dehiscence or infection. Such adverse effects would be reasons for immediate euthanasia.

Non-surgical harvesting of immature (stage 0) *Xenopus* eggs:

This method involves injection of human chorionic gonadotropin (HCG) - 500 IU into the dorsal lymph sac of female animals. Females should begin laying eggs 12 hours after the HCG injection and the harvesting process involves gently squeezing eggs from females at that interval. When properly performed by trained 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.

Any deviation from these guidelines requires adequate scientific justification and approval by IACUC before the procedures are performed.

References:

NIH Office of Animal Care and Use (2005) Guidelines for Egg and Oocyte Harvesting in *Xenopus laevis* (<http://oacu.od.nih.gov/ARAC/oocyte.pdf>).