Guidelines for Egg and Oocyte Harvesting in *Xenopus laevis*

Amphibian oocytes are used for studies in molecular biology, embryology and biochemistry. 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.

Stage I-VI oocytes are obtained by surgical laparotomy. Multiple surgeries on a single animal may be justified considering the reduction in the total number of animals used over the long term. However, the total number of animals used must be considered relative to the pain or distress experienced by an individual animal.

1. The total number of laparotomies should be limited and will depend on the condition 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 five recovery surgeries (the 6th would be terminal) per animal are acceptable. Additional survival surgeries should have approval of the individual ACUC.

2. 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. Choice of anesthetic agent should be based on familiarity of the investigator with its use and in consultation with the attending veterinarian. Cooling and hypothermia are not recommended as an adjunct to MS-222 anesthesia.[1]

3. Surgeries should be done as aseptically as practical including the use of sterilized instruments and powderless gloves. Instruments should be sterilized by autoclaving or using a glass-bead sterilizer between animals. 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. Use of aseptic technique may improve oocyte quality by preventing cross contamination of the sample by frog skin bacterial flora.[2]

4. Currently, rinsing with a steady stream of 0.9% sodium chloride alone or 0.5% povidone iodine (with a final rinse of 0.9% saline) for at least 5 seconds is recommended for the preparation of the surgical site.[3] The use of 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. Chlorhexidine is not permitted. [3]

   Use of aseptic technique may improve oocyte quality by preventing cross contamination of the sample by frog skin bacterial flora.[2]

   The decisions regarding performing single surgical site skin preps are left to the discretion of the NIH scientist in consultation with his/her IC veterinary staff and with approval by the ACUC.

5. Careful selection of suture materials and patterns can minimize post-surgical complications.[4]

Monofilament sutures such as nylon have been shown to cause less inflammatory reaction in *Xenopus* skin. Closure in two layers (muscle layer and skin) is recommended particularly for surgical approaches that are off of the midline. Sutures should be removed no later than 2 weeks.

6. There is a paucity of information regarding the appropriate use and dosing of analgesics in *Xenopus* laevis.[5] However, there is some evidence that analgesics commonly used in other species or for other applications may have limited efficacy in frogs following the oocyte harvest procedure. One study reported the administration of flunixin meglumine (25 mg/kg via the dorsal lymph sac) results in analgesia in *Xenopus* and other frog species[6], and another noted that Meloxicam (0.1 mg/kg IM once daily) has also been shown to provide analgesia in other species of frogs.[7] Neither drug has been associated with negative side effects. Administration of analgesic agents, such as these, should be left to the discretion of the IC veterinary staff in consultation with the NIH scientist.

7. Single housing or small group housing for several days after surgery should be considered as part of the post-surgical care of animals undergoing laparotomy. Frogs should be monitored daily during this period for appetite as well as for any complications such as dehiscence or infection. Such adverse effects would be reasons for immediate euthanasia.

8. 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 one month between laparotomies, ensuring full recovery and healing of incision site. Shorter resting periods may be appropriate if only small amounts of tissue are harvested. Recovery time of less than one month should have approval of the IC's ACUC.

9. Investigators should maintain records in accordance to animal facility standard operating procedures and consider methods to individually identify or group 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.

References:

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