Guidelines for Egg and Oocyte Harvesting in *Xenopus* Species

Amphibian eggs and oocytes are used for studies in molecular biology, embryology, and biochemistry. *Xenopus* spp (*X. laevis* and *X. tropicalis*) are commonly used for such studies. Induction of ovulation and/or gentle squeezing of females is used to collect unfertilized eggs while oocytes (immature eggs not capable of being fertilized) are collected using a surgical approach. The surgical approach is commonly done in *X. laevis*.

Unfertilized Egg Collection Methods:

1) Stripping: Eggs are collected by gently squeezing females which have been injected with human chorionic gonadotropin (pharmaceutical grade HCG) or a combination of pharmaceutical grade pregnant mare serum gonadotropin (PMSG) and HCG. [7, 9, 14] When properly performed using gentle massaging motions by technically proficient research personnel, female *Xenopus* are not harmed by the egg stripping procedure and can be used again after a rest period of at least two months. It is recommended to only perform this procedure for a total of 6 times per female. In the event of an adverse clinical condition, veterinary attention or immediate euthanasia should be completed.
2) Egg laying and collection: Females are primed with a combination of PMSG and HCG then placed in a static tank with water overnight. Eggs are collected from the water after a period of time and the frogs returned to normal housing.

Surgical Laparotomy to obtain oocytes:

In *Xenopus*, oogenesis is divided into six stages (I-VI) and oocytes are obtained by surgical laparotomy from anesthetized female *X. laevis*. The selection of the donor female depends on the stage of oocytes required.[5] Sexually mature females yield high number of stage V-VI and juvenile females have more stage I-II oocytes.[3, 5] The surgical laparotomy to harvest oocytes causes minimal pain/distress and minimal postsurgical complications.[3]

1) Multiple surgeries on a single animal must be scientifically justified and approved by the individual's ACUC.[2] If multiple surgeries are approved, the total number of laparotomies should be limited and will depend on the condition of the animal, the quality of the oocytes, the life span of the animal and the duration of egg production. A maximum of two recovery surgeries one on the left ovary and one on the right ovary per animal is recommended if multiple surgeries are approved. A 3rd non-survival surgery collecting all oocytes may be performed after full recovery from the second surgery.

   Adequate recovery time should be allowed between laparotomies. A minimum of 1 month recovery between surgeries should be allowed when repetitive laparotomies are approved to ensure full recovery and healing of the incision site.
2) Surgeries should be performed by trained personnel using appropriate anesthesia and analgesia. Anesthetic options include pharmaceutical grade buffered tricaine methane-sulfonate (MS-222), etomidate, benzocaine, or isoflurane. [4, 10, 11] (Cooling and hypothermia are not recommended as an adjunct to MS- 222 anesthesia. Analgesia may include the administration of flunixin meglumine (25 mg/kg via the dorsal lymph sac), butorphanol (25 mg/kg intracoelomic), xylazine (10 mg/kg intracoelomic) or meloxicam (0.1 mg/kg IM once daily).[1, 10, 12]

3) Surgeries should be done with aseptic technique including the use of sterilized instruments and powderless gloves. Instruments should be sterilized by autoclaving or using a glass-bead sterilizer since residual cold sterilant might expose the permeable amphibian skin to toxic chemicals. Use of aseptic technique will improve healing and may improve oocyte quality by preventing cross contamination of the sample by frog skin bacterial flora.[3]
4) Preparation of the skin at the incision site must be specified in the ASP and developed in consultation with the IC veterinary staff. Chlorhexidine or other detergents are not recommended. Chemical agents may disrupt the normal skin flora of the patient and the constant mucous production of *Xenopus* skin makes any sterilization effort transient. The protective mucous layer contains magainins, antimicrobial agents that help protect the animal.[6] Preparation of the surgical site should be considered with a rinse of 0.9% sodium chloride alone or 0.5% povidone iodine (with a final rinse of 0.9% saline) as a steady stream for at least 5 seconds. [8]
5) Careful selection of suture materials and patterns can minimize post-surgical complications.[13] Closure in two layers (muscle layer and skin) is recommended particularly for surgical approaches that are off of the midline. Absorbable suture should be used to close the muscle layer. If non-absorbable sutures are used to close the skin, any residual sutures should be removed within 2-3 weeks following surgery.
6) Single housing or small group housing for several days after surgery may 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.
7) Investigators must maintain surgical records in accordance with animal facility standard operating procedures. Consider methods to individually identify or group animals by surgical date and number of prior surgeries, to track how many surgeries are performed on a given animal. Individual identification methods may include color-coded beads sutured to the animal's skin, subcutaneous dyes or a photography log of the unique patterns on each animal's dorsum.

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