

## 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:

Unfertilized eggs are collected by gently squeezing eggs from 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. Some protocols for priming females are described below, however the list is not all inclusive:

*X. laevis* may receive a single dose of 500-800 IU HCG (1000 U/ml) injected into the dorsal lymph sac or are primed using 0.5ml of 200 U/ml PMSG followed by 0.5ml of 1000U/ml HCG 2-3 days later.<sup>6; 8; 12</sup> *X. laevis* females should begin laying eggs 12-14 hours after the HCG injection.

*X. tropicalis* receive a double dose of HCG injected into the dorsal lymph sac separated by ~12-16 hours [primed with low dose 15-20 IU (100 IU/ml) and boosted with 100-200 IU HCG (1,000 IU/ml)]<sup>6; 12</sup> *X. tropicalis* females should begin laying eggs 3 hours after the boosting HCG injection.

When properly performed by technically proficient research personnel, female *Xenopus* are not harmed by the egg squeezing (stripping) procedure and can be used again after a rest period of 3-6 months. It is recommended to only perform this procedure for a total of 6 times per female. Facial petechiations may occur in females *Xenopus*, therefore gentle massaging motions mimicking the male frog mating behavior is recommended. In the event of an adverse clinical condition, veterinary attention or immediate euthanasia should be completed.

### 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.<sup>4</sup> Sexually mature females yield high number of stage V-VI and juvenile females have more stage I-II oocytes.<sup>2; 4</sup> The surgical laparotomy to harvest oocytes cause minimal pain/distress and minimal postsurgical complications.<sup>2</sup>

1. Multiple surgeries on a single animal must be scientifically justified and approved by the individual's ACUC.<sup>1</sup> 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 five recovery surgeries (the 6<sup>th</sup> would be terminal) per animal is recommended if multiple surgeries are approved.

Adequate recovery time should be allowed between laparotomies. A minimum of 3 months recovery between surgeries should be allowed when repetitive laparotomies are approved to ensure full recovery and healing of the incision site.<sup>11</sup> 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. Shorter resting periods may be appropriate if only small amounts of tissue are harvested and have approval of the IC's ACUC.

2. Surgeries should be performed by trained personnel using appropriate anesthesia such as pharmaceutical grade tricaine methane-sulfonate (MS-222). MS-222 has human safety concerns and therefore personnel must follow NIH safety guidelines when mixing it into solution. MS-222 solution should be buffered to a neutral pH of 7.0 for animal welfare and personnel should follow facilities standard of operation when buffering the solution.<sup>3</sup> 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.
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 sterilant should be avoided so 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.<sup>2</sup>
4. 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.<sup>5</sup> Therefore, 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.

General guidelines for skin prep are to avoid chemical surgical preps since they could be systemically absorbed and adversely affect the frog. Chlorhexidine is not permitted nor scrubs containing soaps or detergents. 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.<sup>7</sup>

5. Careful selection of suture materials and patterns can minimize post-surgical complications.<sup>10</sup> Closure in two layers (muscle layer and skin) is recommended particularly for surgical approaches that are off of the midline. Absorbable suture (PDS or Vicryl) should be used to close the muscle layer. Non-absorbable monofilament sutures (nylon) cause less inflammatory reaction in *Xenopus* skin and should be used to close the skin. Sutures should be removed within 2-3 weeks following surgery.
6. There is a paucity of information regarding the appropriate use and dosing of analgesics in *X. laevis*. 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<sup>6</sup>, and another noted that Meloxicam (0.1 mg/kg IM once daily) has also been shown to provide analgesia in other species of frogs.<sup>9</sup> 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. Investigators must maintain surgical records in accordance with animal facility standard operating procedures. Consider methods to individually identify or group animals, which receive surgery, to track how many surgeries are performed on a given animal. Identification methods 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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