

Version: 1.4 Effective Date: March 13, 2024	MULTIPLE PARTIAL OVARIECTOMIES ON <i>XENOPUS SPECIES</i> (MPOX) POLICY	Supersedes Document Dated: <i>10/11/2023</i>
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I. Background

Investigators may need to identify frogs, specifically *Xenopus spp.*, producing oocytes of sufficient quality for the proposed experiments. For example, oocytes are sometimes used for the transcription and/or translation of exogenous DNA or RNA templates. Oocytes from different frogs have been observed to have very different efficiencies of DNA transcription or RNA translation. The quality of oocytes from any given frog is usually constant over several months, so that a frog that is found to be of good quality (i.e., one that produces oocytes showing acceptable quality in the relevant transcriptional or translational assays) can be re-used with an increased assurance of high-quality oocytes. Therefore, investigators may need to perform multiple partial ovariectomies in *Xenopus spp.* (MPOX).

II. Policy

Investigators may perform multiple partial ovariectomies on *Xenopus spp.* if there is a clear and compelling scientific rationale for why multiple partial ovariectomies in *Xenopus* (MPOX) are justified for their research.

III. Responsibilities

- A. Animal Care and Use Committee (ACUC) – MPOX must be scientifically justified in the animal use protocol and approved by the ACUC. Tracking procedures and record keeping may be requested for review during semi-annual inspections.
- B. Office of Laboratory Animal Care (OLAC) – OLAC animal care personnel should ask for clarification from the lab if tracking procedures are not clear.
- C. Principal Investigator (PI) – Surgeries must be performed by trained personnel using appropriate anesthesia and analgesia. The PI is responsible for ensuring that all lab personnel performing MPOX or overseeing frogs that have undergone MPOX understand and abide by this policy.

IV. Required Procedures

- A. A description of the surgeries and related procedures as well as a clear and compelling scientific rationale for why MPOX are justified for their research must be included in the PI's Animal Use Protocol (AUP). Conservation of animal numbers (except for endangered species), economy of animal husbandry, cost of animals, and similar considerations are not acceptable rationale.
- B. Maximum number of surgeries and inter-surgical intervals

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1. No more than six (6) partial ovariectomies may be performed per frog, with a minimum interval between surgeries of no less than three (3) months.
2. The sixth and final ovariectomy must be a terminal surgery.

C. Tracking and Record Keeping

1. If the rationale for performing MPOX is to identify and re-use individual frogs known to produce optimal oocytes, the investigator must provide a plan for:
 - a) Tracking these frogs;
 - b) Tracking the number of surgeries performed on each frog; and,
 - c) Ensuring the minimum interval of three (3) months between surgeries.
2. Procedures for tracking can include, but are not limited to, segregation of operated animals into different post-surgical holding tanks, or physical marking of individual animals. Physical identification may include, but is not limited to, freeze branding, tattooing, color coded beads, photography of the unique markings on each animals' dorsum, implantation of sterile electronic personal information transponders (PIT), or elastomer tags. The identification method must be described in the approved AUP.
3. Investigators must document the number of surgeries performed, the dates upon which and by whom they were performed, and the postsurgical monitoring for frogs subjected to MPOX. The methods used to document the number of surgeries must be described in the AUP. These records must be readily available for review during inspections of the animals and/or their housing area by representatives from OLAC, the ACUC, or external regulatory or accrediting agencies.

D. Surgery and Anesthesia

1. All MPOX must be performed aseptically and in accordance with campus surgery guidelines. The surgical site must be gently swabbed or rinsed with sterile saline. Harsh soaps or scrubs with detergents should not be used on amphibian skin.
2. Frogs must be adequately anesthetized prior to surgery with an anesthetic regimen described in the AUP. Anesthetic options include pharmaceutical grade buffered tricaine methane-sulfonate (MS-222), etomidate, benzocaine, or isoflurane. (Cooling and hypothermia are not recommended as an adjunct to MS- 222 anesthesia. Anesthetic depth must be checked by toe pinch every 15 minutes and prior to start of surgery. This must be recorded in the anesthetic record. Please refer to *ACUC Recordkeeping Guidelines for Surgical*

Procedures on Laboratory Animals for additional information.

3. Hypothermia is not an acceptable method of immobilization (2013 AVMA Guidelines for Euthanasia). Examples of acceptable anesthetics include pharmaceutical grade solutions of benzocaine or buffered tricaine (MS-222). Anesthetic method must be recorded in the animal's record.
4. Peri-operative Analgesia: Analgesics must be administered for this procedure. 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). If analgesics cannot be used, scientific justification must be described in the AUP.
5. Nares must not be submerged during anesthesia and recovery. Skin should be kept moist throughout the procedure.
6. Incisions should be closed via sutures. Absorbable and nonabsorbable sutures are acceptable.

E. Post-operative Monitoring

1. Frogs must be monitored by laboratory personnel daily for at least 48 hours. Frogs must then be monitored every 2-3 days for at least 2 weeks. Post-operative monitoring must be recorded in each animals' surgical record.
2. Incisions should be monitored until healing is complete. Nonabsorbable sutures must be removed in 2-3 weeks.
3. Labs should also consider collecting post-operative measurements (e.g., weight, feeding response, response to touch, changes in blood cortisol) during the days following surgery and compare to pre-op baseline.
4. Euthanasia of the animal must be recorded if animal is undergoing terminal surgery.

V. References

- A. Institute of Laboratory Animal Research (ILAR). National Research Council (2011). [Guide for the Care and Use of Laboratory Animals \(8th Edition\)](#). Washington, D.C.: The National Academies Press.
- B. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition, pg. 94
- C. Reference UNC: <http://research.unc.edu/offices/iacuc/policies-procedures/>
- D. NIH Guidelines for Egg and Oocyte Harvesting in Xenopus Species [https://oacu.oir.nih.gov/system/files/media/file/2022-03/b11 xenopus oocyte collection guideline.pdf](https://oacu.oir.nih.gov/system/files/media/file/2022-03/b11_xenopus_oocyte_collection_guideline.pdf)
- E. Philips BH, Crim MJ, Hankenson FC, Steffen EK, Klein PS, Brice AK,

Carty AJ. 2015. Evaluation of Presurgical Skin Preparation Agents in African Clawed Frogs (*Xenopus laevis*). *Journal of the American Association for Laboratory Animal Science* 54:788-798.

- F. Villeneuve P, Esnault G, Benoit E, Molgo J, Araoz R. A monitoring study of repetitive surgical oocyte harvest in *Xenopus laevis*, pp. 173-178. Meeting on Toxinology-Toxins and ion transfers French Society of Toxinology.