UNIVERSITY STANDARD

UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL
STANDARD ON SURVIVAL SURGERY IN AMPHIBIAN OOCYTE HARVEST

Introduction

PURPOSE
To describe aseptic technique for amphibian oocyte harvesting survival surgeries. This Standard allows for five survival surgeries and one non-survival surgery to be performed on a single amphibian.

SCOPE OF APPLICABILITY
All researchers involved with harvesting amphibian oocytes in live animal studies.

The UNC-CH IACUC expects that anyone involved in animal work at the University will comply with this Standard. Requests for exceptions to this Standard must be reviewed and approved by the IACUC.

Standard

The IACUC recommends:

• fasting the amphibian 12 - 24 hours prior to surgery to prevent food regurgitation.
• allowing a minimum recovery time of 16 weeks between surgeries¹.
• alternating surgery sites for oocyte harvest.

Anesthesia:
*Tricaine Methanesulfonate (MS222),⁵ buffered to neutral pH with sodium bicarbonate, is commonly used to anesthetize amphibians. Water from the same water source as the amphibian’s holding tank should be used, if possible, as the amphibian is acclimated to the water quality and temperature. When working with MS222, it is required that personnel wear gloves at all times, and work in a fume hood when handling MS222 in powder form, to protect against accidental absorption through the skin. Please consult the EH&S SOP for MS-222 before using.

1. Place the amphibian in an induction chamber of buffered MS222 and observe closely (ensure the chamber is secured to prevent the amphibian from jumping out.)
2. Tilting the induction chamber to keep the nares out of the water helps prevent drowning.
3. Determine the depth of anesthesia by toe pinch, eye blink reflex, and/or halting of gular (throat) movement.
4. Once the amphibian has reached a surgical plane of anesthesia, remove the amphibian from the induction chamber.
5. If needed, the appropriate depth of anesthesia may be maintained for surgery by dripping some of the liquid anesthetic on the amphibian’s body or by placing a nonabrasive material, soaked in MS222, in direct contact with the amphibian’s skin.

Aseptic Techniques:

1. Place the amphibian on its back, and cover the non-surgical area with nonabrasive sterile drapes moistened with water over part of the amphibian’s body to keep the skin moist.
2. Avoid all soaps, scrubs, alcohol and full strength solutions, which can injure the protective mucous layer of the skin, or dry out the skin.
3. Using a nonabrasive, sterile material, prepare the incision site by applying a single layer of very dilute
   a. 0.5% povidone iodine solution followed by a rinse with 0.9% sterile saline
4. All instruments must be sterilized prior to the surgery, preferably by autoclaving or by hot bead sterilization. Cold sterilants should be avoided to prevent accidental introduction of toxic chemicals to the surgical site or the amphibian skin. Acceptable sterilants are listed under the IACUC approved University Standard for Aseptic Rodent Surgery. If cold sterilants are used, make sure to rinse thoroughly with sterile saline or sterile water.
5. The surgeon should wear a face mask and sterile gloves.
6. Change gloves between animals.
7. Close the muscle and skin layers separately. The skin layer should be closed with a monofilament suture, e.g. nylon.

Animal Recovery:

1. Gently rinse the amphibian in a small amount of water from the home tank to assist recovery from anesthesia.
2. Place the amphibian in a shallow container with water. Using water from the amphibian’s home tank helps reduce stress.
3. Tilting the induction chamber to keep the nares out of the water helps prevent drowning.
4. Return the amphibian to the home tank after full recovery from anesthesia.
**Record Keeping**

1. Keep precise surgical records on each animal, including information such as:
   a. surgery date
   b. all drug(s) administered (anesthetics and analgesics)
   c. route of administration, & documentation of surgical plane of anesthesia reached (ex. lack of toe pinch response)
   d. administered dose
   e. post-surgical monitoring performed
   f. complications, if any
   g. date and method of euthanasia.

**Sterile gloves are not required if the surgeon only touches the incision site with sterile instruments. To determine if your surgery requires the use of sterile gloves, please contact The Office of Animal Care and Use at 966-5569 for assistance.**

**References:**

1. Guidelines for Egg and Oocyte Harvesting in Xenopus laevis, NIH, ARAC.
2. Guidance on the Housing and Care of the African Clawed Frog, Royal Society for the Prevention of Cruelty to Animals
5. Pharmaceutical grade Tricaine Methanesulfonate (MS222) may be purchased from the following company: Tricaine-S: Western Chemical Inc.

**EXCEPTIONS**

If your experimental procedure requires a significant deviation to this Standard Operating Procedure (SOP), please amend your application(s) to include Addendum 8.0 Request for Exception to Policy and indicate the following: a description of the exception; the rationale (provide scientific justification and/or justification based on animal welfare); the potential adverse effects/clinical signs resulting from the exception; and specify which (and the total number of) animals in the approved protocol that will be affected. The IACUC will review your request at the next monthly meeting.
Definitions

IACUC: Institutional Animal Care and Use Committee
OACU: Office of Animal Care and Use
DCM: Division of Comparative Medicine
NIH: National Institutes of Health
ARAC: Animal Research Advisory Committee
JAALAS: Journal of the American Association for Laboratory Animal Science
Oocyte: a cell in an ovary that may undergo meiotic division to form an ovum
University Standard: The minimum acceptable limits or rules used to achieve Policy implementation, enforceable by the IACUC.

Related Requirements

UNIVERSITY POLICIES, STANDARDS, AND PROCEDURES

For more detailed guidance, please refer to the University Policy on the Care and Use of Vertebrate Animals for Research, Training and Teaching Purposes.

Contact Information

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<th>Subject</th>
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<tbody>
<tr>
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Important Dates

- Effective Date and title of Approver: 06/24/2005; UNC IACUC
- Revision and Review Dates, Change notes, title of Reviewer or Approver: revised 03/11/2011, revised 02/2018; UNC IACUC

Approved by: UNC IACUC

Dr. Mitchell Picker
UNC IACUC Chair