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POLITICA Y GUIAS SOBRE CIRUGIA EN ANIMALES DE LABORATORIO

Estimado doctor Aymat:

Con el propósito de que nuestros investigadores, pudieran como parte de sus protocolos de investigación, realizar procedimientos quirúrgicos en animales de laboratorio el Recinto tenía varias políticas separadas. Después de actualizar las políticas sometemos para su consideración la actualización de la Política y Guías para Cirugías en Animales de Laboratorio. Con esta política nos aseguramos de que nuestros investigadores y su personal tengan unas guías básicas y puedan cumplir con las regulaciones vigentes.

Por tanto el IACUC somete esta política para su revisión y firma.

Gracias anticipadas,

Elizabeth Rivera, DVM
Presidenta IACUC

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SURGERY POLICY AND GUIDELINES

I. Introduction

The Medical Sciences Campus is a PHS Fund receiving institution, USDA registered research facility, and AAALAC accredited, it has the responsibility to provide proper treatment to animals involved in research. According to the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training (The Principles) incorporated in the Public Health Service Policy on Humane Care and Use of Laboratory Animals (PHS Policy) under the mandate of the Health Research Extension Act of 1985, Public Law 99-158, "Animals in Research" and The Guide for the Care and Use of Laboratory Animals: Eighth Edition (The Guide) our institution among these treatments must also provide appropriate pre-surgical and post-surgical veterinary medical care. USDA Policy # 3: Veterinary Care also states that the Animal Welfare Act (AWA) requires that all regulated animals be provided adequate veterinary care. Both of these acts require the appropriate use of tranquilizers, sedatives, analgesics, and anesthetics.

Hence, this policy ensures that surgeries are completed using the basic rules of asepsis, gentle tissue handling, anesthetic maintenance, and proper post-operative care, and are carried out in accordance with applicable governmental regulations stated above. In addition, good animal care and use will optimize research results and minimize variability, thus making it possible to use fewer animals.

II. Definitions

A. **Dedicated Surgical Facility** - areas that are set up to be cleaned and maintained in an aseptic condition, and are not used for other purposes when they are not being used for surgery.

B. **Aseptic Technique** - practices and procedures used to reduce microbial contamination to the lowest possible level.

C. **Survival Surgery** - an operative procedure after which the animal recovers from anesthesia. Whether a procedure is major or minor will be evaluated on a case-by-case basis by the veterinarian and IACUC.

D. **Major survival surgical procedure** - The Guide states that a ***major survival surgery*** penetrates and exposes a body cavity or produces substantial impairment of physical or physiologic functions (such as laparotomy, thoracotomy, craniotomy, joint replacement, and limb amputation) or involves extensive tissue dissection or transection. The AWA similarly defines a major operative procedure, as any surgical intervention that penetrates and exposes a body cavity or any procedure that produces permanent impairment of physical or physiological functions.

E. **Minor survival surgical procedure** - The Guide states that a ***minor survival surgery*** does not expose a body cavity and causes little or no physical impairment (such as wound suturing; peripheral – vessel cannulation: and most procedures routinely done on an outpatient basis in veterinary clinical practice). Minor procedures are often performed under less-stringent conditions than major survival procedures but still require aseptic technique, sterile instruments, appropriate anesthesia and training. Although laparoscopic procedures are often performed on an "outpatient" basis, appropriate aseptic technique, instruments, anesthesia and analgesia are necessary. Whether a laparoscopic procedure is deemed major or minor will be evaluated on a case-by-case basis by the veterinarian and IACUC.

F. **Multiple Survival Surgery** - Animal recovers from initial surgery (major or minor) and is subsequently re-anesthetized for one or more survival surgical procedures (major and/or minor) related to the proposed study.

1. Multiple **major** surgical procedures - No animal may be used in more than one major operative procedure from which it is allowed to recover, unless, 1) included in and essential components of a single research protocol 2) justified for scientific reason, 3) required as routine veterinary procedure or to protect the health or well-being of the animal as determined by the veterinarian, the PHS or the USDA guidelines.

2. Multiple **minor** surgical procedures - Minor procedures that may cause substantial post operative pain or impairment must be scientifically justified if performed more than once in a single animal.

3. Cost savings alone is not a justification for multiple survival surgeries.

G. **Terminal (non-survival) Surgery** - a procedure in which the animal is euthanized prior to recovery from anesthesia. It is not necessary to follow all the techniques described below. However at a minimum, the surgical site should be clipped, the surgeon should wear gloves, and the instruments and surrounding area should be clean. Terminal Surgery of long duration will be evaluated on a case-by-case basis by the veterinarian and IACUC.

III. General Requirements for all species

A. **Training** - Research personnel conducting surgical procedures must have appropriate training prior to performing surgery. Training is available at no charge through the IACUC's Animal Use Training Sessions

B. **Pre surgical planning** - Should include input from all the surgical team, it should specify the requirements for postsurgical monitoring, care, and recordkeeping, including the personnel who will perform these duties.

C. **Expired materials** - may be used in terminal surgeries where the animal is anesthetized and euthanized without recovery if such use does not adversely affect the animal wellbeing or compromise the validity of the study, ***EXCEPT*** analgesics, sedatives, anesthetics and euthanasia solutions.

D. **Pharmaceutical grades substances** - are expected to be used whenever they are available. These include but are not limited to: compounds, medications, drugs, vehicles and diluents. MSC IACUC recognizes that some substances e.g., test articles, novel compounds and those resulting from compounding process) are only available as non-pharmaceutical grade product. Non pharmaceutical grade substances should only be used in USDA-Covered Species after specific review and approval by the IACUC. The use of non-pharmaceutical grade substances will follow the MSC Non-Pharmaceutical Grade Substances Use Guidelines.

E. **Analgesia** - Analgesia must be provided to animals that are likely to experience post procedural pain; exceptions must be approved by the IACUC. Analgesia must be provided preoperatively unless scientifically justified in the IACUC approved protocol.

F. **Protocol** - All surgical procedures, anesthetics, analgesics, antibiotics or other medications used on animals must be approved by the IACUC and described in the animal use protocol. Personnel performing the surgical procedure must be listed on the animal use protocol and appropriately trained in the technique prior to starting any surgeries. Any divergence from the recommendations in this document must be described in the animal use protocol prior to implementing the deviation.

IV. Requirements and Procedures for USDA-Covered Species Survival Surgery (or Survival Procedures)

A. **Surgical Area** - Aseptic surgery (major and minor) must be conducted in a dedicated facility. Survival surgeries in USDA non-rodent species will be done in the Experimental Surgery Laboratory, located in the 10th Floor of the Guillermo Arbona Building. Surgical facilities used for aseptic surgery must be maintained and operated in a manner that ensures cleanliness and minimizes unnecessary traffic. Interior surfaces must be monolithic and impervious to moisture. Functional components of aseptic surgery include surgical support, animal preparation, surgeon's scrub, operating room and postoperative recovery. A plan to sanitize areas used for other purposes must be in writing and documented when completed. Exceptions to the above criteria, if justified as an essential component of the research protocol, must have IACUC approval. Non-survival surgeries do not require a dedicated facility but must be performed in a clean area, free of clutter, and using standard sanitation practices.

When justified, a PI-managed lab may be IACUC-approved for survival surgery when it is scientifically or technically justified and meets the following conditions:

1. The interior surfaces are monolithic and impervious to moisture (non-porous).
2. OR must be sanitized prior to surgical procedures. When not in use, the OR should be maintained clean and clutter-free.

3. The OR should not be used for primary storage. If needed, items that are not easily sanitized (e.g. non-surgical equipment, card board boxes, general supplies, etc.) should be stored in drawers or cabinets which can be sanitized.

4. Positive air pressure should be maintained (relative to the surrounding areas) to prevent airborne contaminants from entering the OR.

Minor survival procedures (lymph nodes biopsy, skin/masses biopsies, wound closure) can be conducted in a designated area that is not used for other purposes when they are being used for surgery.

B. **Aseptic technique** - must be followed for all survival surgeries. All surgical equipment, implanted material, instruments, and supplies that will come in contact with the surgical site must be sterilized before use in survival surgery.

Personnel taking part in the surgery (members of the surgical team) must wear clean scrubs, appropriate face mask, head covering, close-toed shoes. If available shoe covers should be worn. Must perform a surgical scrub in the surgeon's scrub area; this includes scrubbing both hands, in between fingers, and both forearms with a designated surgical scrub brush with an antibacterial soap (e.g., chlorhexidine or iodophor) for as long as the product indicates prior to initiating the surgical procedure. Must wear sterile gown and gloves using appropriate technique. Limit excessive movement (e.g. foot traffic) to avoid contamination of the surgical area. Rescrub and regown if it is necessary to leave the surgical suite.

Non-surgeons (e.g., anesthetists, surgical assistants) must wear appropriate personal protective equipment (PPE)—at least a disposable cover gown, mask, gloves and head cap. Non-surgeons are defined as those individuals that will NOT touch the animal undergoing the surgical procedure, but they may assist the surgeon.

All instruments must be cleaned and sterilized prior to use on animals for all surgical procedures. Alcohol is NOT a sterilant. Examples of methods of sterilization include steam autoclave, gas (e.g. ethylene oxide), and plasma sterilization. **See Table 1. Instrument Sterilants**. Cold sterilization (e.g. Cidex) of surgical instruments must strictly follow manufacturer instructions. The CDC lists specific cold sterilants and the necessary conditions (e.g., contact times) to be considered a sterilant or a disinfectant. Rinse instruments free from the cold sterilants with sterile water or sterile saline before putting them in contact with animals. **See Table 2. Instrument Disinfectant**. Only sterile instruments can be used for each animal. Therefore, new autoclaved or gas sterilized packs are required for each animal. However certain exemptions may be approved by IACUC. Do not use dull or rusted surgical instruments or those not manufactured for surgical use. When sterilizing by autoclaving or using plasma sterilizer, surgical packs should contain a sterilization indicator, the date of sterilization and expiration date for the pack. Protected storage of sterilized items is required; integrity of sterile packaging must be examined prior to use. Instruments must be replaced and the hands re-gloved following contact with non-sterile surfaces.

Hair/fur must be removed and the surgical site scrubbed three times with a skin disinfectant, such as chlorhexidine or iodophors, alternating each disinfectant scrub with a scrub of sterile water or 70% isopropyl alcohol. The surgical site must be draped by placing and securing sterile drapes over the entire animal and the operating table.

For non-survival surgeries, asepsis is not required however; the surgeon must wear clean gloves, a clean gown or lab coat, and use clean instruments.

C. **Anesthesia** – According to The Guide (pg. 119), *"Careful surgical monitoring and timely attention to problems increase the likelihood of a successful surgical outcome"*. Anesthetic selection is evaluated during the IACUC protocol approval process and additional veterinary consultation is available at all times. Assessment of the animal's physiologic condition and plane of anesthesia (immobilization versus surgical plane) must occur at least every 10 minutes throughout the procedure. At a minimum, vital signs such as heart and respiratory rates must be recorded.

D. **Analgesia** - Analgesia must be provided to animals that are likely to experience post procedural pain; exceptions must be approved by the IACUC. Analgesia must be provided preoperatively unless scientifically justified in the IACUC approved protocol. Unless the contrary it is established, investigators should consider procedures that cause pain or distress in human beings may cause pain or distress in other animals as well. Investigators are responsible for the assessment and management of pain in their research animals and must describe a plan for pain management in their IACUC protocol.

E. **Post Op Care** - Post-operative animal care must include monitoring, record keeping, and documentation of any treatments given. Study team personnel must provide the postoperative care unless arrangements have been made with the ARC in advance. The frequency of observation is dependent on the phase of recovery. The veterinary staff must be informed of any post-operative complications.

1. Continuous monitoring (heart rate, respiratory rate, and temperature) is required and supportive care must be provided until the animal can maintain sternal upright positioning and has been extubated (removal of the endotracheal tube).
2. Following extubation, animals must be monitored every 15 minutes until physiologic parameters (heart rate, respiratory rate, +/- temperature) have returned to levels indicating recovery from anesthesia.
3. Daily observation must be done until all incisions have healed. Special attention should be made to physiologic functions as well as behavioral signs of post-procedural/operative pain, infection, and wound dehiscence. Non-absorbable sutures and staples must be removed at 7-14 days. A daily notation in the animal record is sufficient for record keeping purposes.

F. **Record Keeping** - Investigators must maintain accurate records of anesthesia, surgery, and post-operative care, including analgesic administration. These records serve as documentation that the procedures were conducted humanely by appropriately trained individuals. The records should be completed at the time of the surgical procedure and provide sufficient information to allow the reader to determine what was done to the animal, when it was conducted, and by whom.

Anesthesia and post-operative care records must provide documentation of animal evaluation. Surgical records must document the procedures performed, date, and identify the surgeon and anesthesiologist. Records must be available during the semiannual IACUC inspection and per federal guidelines (AWA), the principle investigator is required to retain these records for a minimum of three years after the completion of the research activity.

For non-surgical anesthetic events (i.e. imaging) lasting more than 30 minutes using USDA covered species (non-rodents) the described requirements for anesthesia and post-operative care monitoring and record keeping must be followed.

V. Requirements and Procedures for Rodent Species (Rat and Mouse) Survival Surgery (or Survival Procedures)

A. Surgical Area

1. According to The Guide (pg. 144), "For most survival surgery performed on rodents and other small species...the space should be dedicated to surgery and related activities when used for this purpose, and managed to minimize contamination from other activities conducted in the room at other times." .

a) The surgical area should be a room or a portion of a room that is easily sanitized and not used for any other purpose during the time of surgery.

b) Clean and disinfect the surface upon which the surgery will be performed with an approved environmental disinfectant before beginning the surgical procedure.

c) Laboratory benches in front of open windows or next to doors exposed to air currents, where contamination by dust and/or foreign particles is difficult to control should not be selected to serve as rodent surgery tables. Surgical facilities should be sufficiently separated from other areas to minimize unnecessary traffic and decrease the potential for contamination.

B. Aseptic Technique

1. The goal of aseptic technique is to reduce the possibility of microbial contamination of the incision and exposed tissues thus preventing post-surgical infection. No single technique, practice, germicide, or piece of equipment will achieve this objective. Rather, proper aseptic technique is dependent on numerous practices that require input and cooperation of all personnel within the operating area.

2. Aseptic technique results in decreased inflammation and gentle tissue handling results in decreased catabolism and enhances recovery and reduces postoperative complications, Infections in rodents can be sub-clinical, but still affect the behavior and/or physiology of the animal. Prevention of infection improves the welfare of the animal and eliminates a source of uncontrolled variation in the experimental results.

3. Preparation of the Surgical Supplies:

a) Surgical Instruments: Use prepackaged aseptic surgical supplies whenever possible. **Initial steam sterilization (autoclaving), plasma vapor sterilization, or ethylene**

oxide sterilization (for heat or pressure sensitive items) is required for all surgical instruments and items to be implanted.

b) Drapes

(1) Used for wrapping the instrument packs and/or creating the sterile field around the incision site.

(2) Sterile impermeable drape such as disposable paper drapes are recommended in order to prevent contamination of the surgical site with organism of the outside of the disinfected area.

(3) Drapes can be cloth, paper, sterile stockinettes, 3M™, Steri-Drape™. Drapes must be changed between each animal.

4. Preparation of the Animal

a) Fasting is not required for rodents due to their high metabolic rate. Fasting longer than 6 hours must be scientifically justified in the approved IACUC protocol.

b) Apply ophthalmic ointment (eye lube) to the animal's eye to prevent corneal damage.

c) Skin Disinfection

(1) Hair must be removed from the surgical site prior to disinfecting the skin. Enough fur should be shaved from the incision site to prevent wound contamination, but care should be taken not to remove excessive fur as to interfere with the animal thermoregulation.

(2) Incision site **must** be disinfected. A recommended skin disinfectant such as chlorhexidine or iodine scrub alternated with warmed saline, sterile water, or alcohol must be used a minimum of three times *after* removal of visible debris. Each step iodine-alcohol-iodine should be performed in a concentric pattern, beginning at the incision site to the periphery of the surgical field.

d) The sterile drape is then placed around the surgical site after the skin been appropriately disinfected.

5. Preparation of the Surgeon

a) Wash hands thoroughly with a disinfecting soap such as chlorhexidine or iodine based surgical scrubs or 3M Avaguard^®^ hand antiseptic.

b) The surgeon and all individuals participating or present in the surgery must wear a mask, a surgical bonnet, sterile or clean gloves, and a clean scrub top, clean disposable PPE gown, or clean lab jacket during the surgical procedure.

(1) Clean gloves include unused standard latex or nitrile lab gloves stored in a sealable bag or container to minimize dust and debris contamination.

(2) Sterile gloves are required if you will be touching the animal or the tips of your sterile instruments with your hands. If you will only be touching the handles of your instruments, then clean, non-sterile gloves are acceptable.

6. Multiple Rodent Surgeries: During operative procedures on multiple rodents, care must be taken to avoid contaminating from one animal to another.

a) Investigators should begin with at least 2 sets of sterile instruments. Preferably separate sterile surgical instruments should be provided for each animal subject.

b) Manipulate the tissues with only the tips of the instruments and avoid handling the tissues directly with your hands, which tend to be more easily contaminated.

c) Between animals, clean the instruments followed by disinfection with a hot bead sterilizer or immersion in liquid sterilants (e.g. Alcide, Clidox, etc.) or disinfectant (e.g. Betadine solution or Chlorhexidine solution) and rinse in sterile saline.

d) No more than 5 animals should be used per pack of sterile instruments and each pack should not be open for more than 2-3 hours.

e) A new sterile drape should be used for each animal.

f) New clean or sterile gloves should be used for each animal. Alternatively, if gloves do not have any gross contamination, surgeons may wipe their gloves with sterile gauze pads soak in sterilant.

g) The surgical area should be cleaned with an appropriate disinfectant between animals.

7. Suture Materials and Wound Closure

a) Surgery in which a body cavity such as the abdomen or thorax has been opened requires a two-layer closure to close the body wall separately from the skin.

b) Select suture material (absorbable vs. non-absorbable; braided vs. monofilament) that is appropriate to the tissues, procedure, and activity of the animal.

c) Wound clips and non-absorbable skin sutures must be removed 7-14 days after placement.

8. Nonsurvival Surgeries

a) For nonsurvival surgeries it may not be necessary to follow aseptic technique but at a minimum, the surgical site should be clipped, the surgeon should wear gloves, and the instruments and surrounding area should be clean.

b) The use of expired drugs is not allowed. Pharmaceutical grade agents must be used unless an exemption is approved by the IACUC.

c) The method of euthanasia should be consistent with the current AVMA Guidelines of Euthanasia and must be listed in the approved IACUC protocol.

C. **Anesthesia and Analgesia**

1. Use an approved agent appropriate for the species AND the procedure.

2. The animal must be maintained in a surgical plane of anesthesia throughout the duration of the procedure.

3. The anesthetic plan should follow the IACUC approved protocol, and must have been discussed with the ARC Clinical Veterinarian prior to approval.

4. Analgesia must be provided preoperatively unless scientifically justified in the IACUC approved protocol.

D. **Peri-Operative, Post-Operative Monitoring and Care**:

1. Animals must be visibly observed and monitored every 15 minutes during recovery from anesthesia until the animal is ambulatory.

2. Cardiovascular and respiratory function should be monitored, which includes respiratory depth. Animals need to be in an appropriate setting such as the use of an indirect thermal blanket, in order to avoid hypothermia.

3. Gas anesthetics usually provide a rapid recovery, while injectable anesthetics may take longer, so an extra care must be needed while monitoring the animal, such as rotate the animal every 10-15 minutes to facilitate respiration and avoid dependent edema.

a) Rodents should be housed individually or monitored closely until completely recovered to avoid cannibalism by cage mates.

b) Monitoring parameters and thermal supplementation should be continued throughout the recovery period.

4. A long Post-operative care includes regular observation of the surgical site, suture removal, dressing changes, observation to return to normal motor function.

5. Post-operative medications including, antibiotics and/or anesthetic reversals should be administered during the early recovery period and according to the approved protocol or the advice of the MSC veterinarians.

a) The date of surgery must be recorded on the animal cage card as well as the date for removal of wound clips or skin sutures.

(1) All wound clips and skin sutures must be removed 7 to 14 days after surgery unless described otherwise in the IACUC approved protocol or as recommended by the MSC veterinarians due to incomplete wound healing.

6. The laboratory staff must examine all post-surgical animals **at least once a day for 7 – 14 days, or until the skin sutures or wound clips are removed.**

a) Daily post-operative monitoring and health status of the animals must be recorded during the post-operative monitoring period and records must be maintained in the post-surgical documentation.

b) Quantity and quality of urine and feces should be monitored to guard against paralytic ileus or irritation of digestive tract and renal shutdown of the urinary tract.

c) Close attention must be provided to avoid self-inflicted trauma.

E. **Supportive Care of Anesthetized Rodent:**

1. In order to minimize the heat loss during anesthesia, thermal blanket (e.g. recirculating warm water blanket) or drape should be placed between the animal and the stainless steel operating table. Heating lamp should be carefully set to avoid burns, if these are the only alternative the investigator has. The use of warm irrigation solution during surgery is highly recommended. Volume deficits can be corrected by SQ injection of warmed saline, LRS or replacement fluid (Normosol).

F. **Record Keeping:**

1. Post-surgical records are required for every rodent that has undergone surgery.

2. Post-surgical records must be contained within the animal room or within close proximity to the animals and readily retrievable for review by laboratory, IACUC, or veterinary personnel at all times.

3. Post-surgical records must be kept and maintained until sutures/wound clips are removed or the animal is euthanized.

a) If skin incisions are not closed with sutures or wound clips, records must remain near the animal for at least 7 days following surgery.

4. Post-surgical records must include anesthetics and analgesics administered for the surgical procedure and after for post-operative care. The records must also include the frequency of monitoring by any laboratory and veterinary personnel during the post-operative period. The following information should be included for both anesthetics and analgesics:

a) Dose

b) Frequency of administration

c) Route of delivery

5. Once the post-operative monitoring period is over, records for mice and rats can be removed from the animal room and stored by lab personnel.

VI. Requirements and Procedures for Other Species Survival Surgery (or Survival Procedures)

A. Amphibians

1. **Preoperative Considerations**

a) To prevent intraoperative regurgitation, it is necessary to establish a fasting period; also an adequate pre-surgical hydration is essential for a successful surgical outcome, therefore soaking the amphibian patient in water for 1 hour before surgery is recommended.

2. **Aseptic Techniques**

a) To avoid damaging their characteristically glandular skin and to avoid potentially zoonotic agents (such as Mycobacterium), it is not appropriate to handle amphibians with bare hands. Non-powdered vinyl or nitrile gloves are preferred, as latex has been shown to cause toxicity in tadpoles and frog embryos.

b) The wet environment required for amphibian surgery makes sterile surgery a challenge, and these procedures are generally considered to be clean-contaminated. Sterile clear plastic (non adhesive) drapes can be helpful. Depending on type of surgery it might be appropriate to institute prophylactic antibiotic therapy before surgery.

c) Surgical scrubs that contain soaps, detergents, isopropyl alcohol, or concentrated iodine products are contraindicated in amphibians. Some publications recommend a site preparation with dilute povidone iodine or chlorhexidine solution, while others suggest that these may disrupt the normal skin flora of the frog due to the semi-permeable nature of the skin.

d) Currently the MSC recommends gentle preparation of the surgical site by removal of gross debris followed by 0.5% povidone-iodine and sterile0.9% saline solution. With a sterile gauze, soaked in the disinfectant apply with direct contact to the surgical site for at least 10 minutes before surgery, then rinse the site with sterile saline.

e) The Principal Investigator should make decisions regarding surgical site preparation in consultation with the MSC veterinary staff.

f) Clean surgical instruments should be wrapped or packed appropriately before being sterilized with high heat and pressure or high heat alone (steam autoclave or drying oven). Instruments should be stored in a dry place in which the integrity of the wrapping or packing material will be maintained for a defined period of time. If multiple surgeries are to be done on different animals, then previously sterilized instruments can be re-sterilized using a glass bead sterilizer.

3. **Anesthesia**

The anesthetic of choice for amphibian surgery is tricaine methane sulfonate (MS-222- is a respiratory irritant; it must be prepare in a Chemical fume hood). Adults generally require the higher concentration.

Recommended doses include 1-2 grams/litter solution of MS-222. Induction times are typically < 30 minutes.

(1) It is important to buffer MS-222 solutions by adding sodium bicarbonate (baking soda) to the water to achieve a pH range between 7.0 and 7.5. When using MS-222 in amphibian patients, a light plane of anesthesia is indicated by the loss of the righting reflex and the corneal reflex. A deep plane of anesthesia is indicated when the withdrawal reflex to deep pain is lost (i.e. toe pinch). Once anesthesia is successfully induced, it is essential to transfer the amphibian patient to fresh water. If the amphibian patient begins to recover before the completion of the surgical procedure, it is advisable to deepen anesthesia by returning the animal to a 50% dilution of the induction solution.

(2) Also, the appropriate depth of anesthesia may be maintained for surgery by dripping some of the liquid anesthetic on the amphibian's body, placing a tube with continues MS-222 water going into the mouth, or by placing a nonabrasive material, soaked in MS-222, in direct contact with the amphibian's skin.

b) Other anesthetic agents may also be acceptable and should be chosen based on investigator experience and consultation with a MSC veterinarian.

c) If inhalant anesthetics are used, such as isoflurane it may be delivered by conventional methods or bubbled directly into the water. These latter techniques, however, make scavenging of waste gas difficult.

d) Also it is possible to perform minor procedures using no more than 1.0 mg/kg total dose of 2% lidocaine as a local anesthetic.

e) Hypothermia is an <u>unacceptable</u> sedation technique for painful procedures.

f) Anesthetic monitoring

(1) It is essential to monitor the amphibian patient during anesthetic induction to prevent drowning. Place the animal with nostrils above the level of anesthetic bath.

(2) If the anesthetic level becomes too deep, it is imperative to rinse the patient with clean well-oxygenated water until the animal recovers. During MS-222 anesthesia, regular respiration will slow or even cease; however, the heart rate is rarely affected.

(3) It is advisable to bubble 100% oxygen into the water during the surgical procedure to assist the amphibian patient's cutaneous respiration. If the heart rate of the amphibian patient drops 20% or greater from baseline, it is necessary to remove the animal from the anesthetic solution and to recover the patient.

(4) In some amphibian patients, it is possible to visualize the heartbeat directly; the use of a Doppler probe is also effective. An additional procedure for monitoring is to attach pulse oximeters to the extremities of the amphibian during surgery.

4. **Surgical Procedures**

a) Skin incisions in amphibian patients are best made with a number 15 or number 11 scalpel blade.

b) Non-absorbable monofilament suture such as nylon (the most common) should be used for skin closure; range from 3–0 or 4–0 to 8-0 suture is generally adequate. Gut suture material, especially chromic gut is to be avoided since it induces a major inflammatory response in amphibians and reptiles.

c) If non absorbable sutures are used (PDS or Vicryl may be used), must be removed within 14 days after surgery.

d) Taper needles are preferable to cutting needles, and it is usually possible to remove sutures after 14 days.

e) In the case of Multiple Survival Surgeries for Oocyte Harvesting in Xenopus Frogs the protocol has to clarify that multiple survival surgeries are required to obtain large quantities of oocytes in the early stages, I-VI, and that there are not suitable alternatives (literature review is required). In addition, scientific justification for the need to performing multiple survival surgeries on an individual frog to collect oocytes is required.

f) The total number of laparotomies for oocyte harvesting must be limited and will depend on the condition of the animal and quality of the oocytes. Up to five (5) survival surgeries (the 6th would be terminal) per animal are acceptable. The investigator should alternate oocyte collection between left and right ovaries and consider rotation of frogs so that the interval between surgeries in any individual is maximized.

g) Adequate recovery time of at least three months between surgeries must be allowed between laparotomies for oocyte harvest. Scientific justification must be provided for request of recovery times of less than three months.

h) Microchips are often used to identify research amphibians, if this is the case the microchip may be placed subcutaneously.

5. **Analgesia** – As the pain perception in frogs is not exactly the same as to mammalian species, post –operative pain may occur and thus analgesia must be provided to animals that are likely to experience post procedural pain; The administration of analgesia is required unless scientifically justified and may be administered either before surgery when the animal has been anesthetized or during recovery.

a. Although, 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, studies have shown that administration of Flunixin meglumine (25 mg/kg via the dorsal lymph sac) results in analgesia in Xenopus and other frog species. Meloxicam (0.1 mg/kg IM once daily) has also been shown to provide analgesia in other species of frogs. Neither drug has been associated with negative side effects.

b. Clinical signs of Pain include agitation, scratching at the incision site, and changes in appetite.

6. **Post Op Care**

a) At the conclusion of surgery, it is essential to transfer the animal to a warm, anesthetic-free bath and to rinse it copiously with fresh, well-oxygenated water.

b) It could be useful to administer fluids they can be administered subcutaneously, or by soaking the animal in a balanced electrolytes solution.

c) It may be necessary to assist the amphibian patient with feeding for a brief period after any surgical procedure.

d) Since sterility is problematic working with amphibians this is why antibiotic therapy is routinely recommended after any surgical procedure.

e) Housing animals singly or in a small groups and monitoring the behavior, appetite and the wound healing for complications such as dehiscence or infection for at least 5 to 7 days post-surgery is required.

f) A Monitoring chart, may be the easiest way the asses animals' health and it should include or take into consideration:

(1) Frequency of monitoring right after surgery and within the next 24 hours, etc. Skin condition, incision condition, inflammation, suture removal, animal activity, application of antibiotics, and application of analgesics.

7. **Record Keeping** -- It is important to maintain records to ensure that proper animal care is occurring. Record keeping should include the number of surgeries per animal and a description of post-operative care and monitoring.

VII. TABLE 1 - RECOMMENDED INSTRUMENT STERILANTS

Note: Always follow manufacturer's instructions for dilution, exposure times and expiration periods.

Agent	Examples*	Comments
Steam sterilization (moist heat)	Autoclave	Effectiveness dependent upon temperature, pressure and time (e.g., 121°C for 15 min. vs 131°C for 3 min).
Dry Heat	Hot Bead Sterilizer Dry Chamber Fast	Instruments must be cooled before contacting tissue. Only tips of instruments are sterilized with hot beads.
Gas sterilization	Ethylene Oxide	Requires 30% or greater relative humidity for effectiveness against spores. Gas is irritating to tissue; all materials require safe airing time
Chlorine	Chlorine Dioxide	Corrosive to instruments. Instruments must be rinsed with sterile saline or sterile water before use.
Glutaraldehydes	Glutaraldehyde (Cidex®, Cetylcide®, Metricide®)	Several hours required for sterilization. Corrosive and irritating. Instruments must be rinsed with sterile saline or sterile water before use.
Hydrogen peroxide-acetic acid	Actril®, Spor-Klenz®	Several hours required for sterilization. Corrosive and irritating. Instruments must be rinsed with sterile saline or sterile water before use.

*The use of common brand names as examples does not indicate a product endorsement.

VIII. TABLE 2 - RECOMMENDED INSTRUMENT DISINFECTANTS

Note: Always follow manufacturer's instructions for dilution, exposure times and expiration periods.

Agent	Examples*	Comments
Alcohols	70% ethyl alcohol 85% isopropyl alcohol	Contact time required is 15 minutes. Contaminated surfaces take longer to disinfect. Remove gross contamination before using. Inexpensive. NOTE: Requires specific IACUC approval for use.
Chlorine	Sodium hypochlorite (Clorox ® 10% solution) Chlorine dioxide (Clidox®)	Corrosive. Presence of organic matter reduces activity. Chlorine dioxide must be fresh. Kills vegetative organisms within 3 min. Corrosive to instruments. Instruments must be rinsed with sterile saline or sterile water before use
Chlorhexidine	Nolvasan® , Hibiclens®	Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses. Instruments must be rinsed with sterile saline or sterile water before use.

*The use of common brand names as examples does not indicate a product endorsement.

IX. Record Samples

EXAMPLE 1: **POST OP CHART** (may vary with species/procedure)

PI: IACUC Protocol #_____

Animal ID: Surgery Date:

	Antibiotic	Analgesia	Fluids	Weight	Comments
Day 1 / /					
Day 2 / /					
Day 3 / /					
Day 4 / /					
Day 5 / /					

Antibiotic: _____: _____ mg/kg Analgesia: _____: _____ mg/kg

EXAMPLE 2: **SURGERY RECORD** (may vary with species/procedure)

PI: Phone: Surgery room/bldg.:

Protocol #: Procedure:

Date performed: Animal #:

Surgeon: Assistant(s):

Pre-operative Physical Examination Notes:

Weight: _____ Temperature: _____

_____

_____

Medications/Drugs Used (drug, dose, route of administration)

Anesthetics: _____

Anesthesia Start Time: _____

Supportive Therapy (fluids, oxygen, etc.):_____

Surgery Start Time: _____ Surgery Completion Time: _____

Description of Operative Procedure and Findings/Complications:

_____

_____

Analgesic Medications (drug, dose, route of administration, frequency, notes):

_____

_____

Surgeon's Signature: _____

X. References

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VoBo:

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