

CHAPTER 11

GUIDELINES FOR RODENT and AQUATIC ANIMAL SURGERY

Rodent Surgery Guidelines:

Background:

Successful surgical outcomes require appropriate attention to pre-surgical planning, personnel training, anesthesia, aseptic and surgical technique, assessment of animal well-being, appropriate use of analgesics, and animal status during the surgical procedure as well as postoperative care.

Post-operative infections in rodents may occur. The infections, which may not be apparent on observation, cause distress to animals and can affect the results of an experimental study. To minimize surgically induced infections in rodents, aseptic surgical procedures must be used in rodents expected to recover from surgery and survive.

Training:

PI's and their staff conducting surgical procedures must have appropriate training to ensure that good surgical techniques are practiced including asepsis, tissue handling, appropriate use of instruments, effective hemostasis, and correct use of suture material. All training techniques must be described and addressed in the UWM Animal Protocol Form. **Note:** if there will be future additional staff that will be performing surgeries, who are not listed as surgeons on the protocol at the time of IACUC approval, the form for Certification of Staff Training for Surgery must be completed and submitted to the animal care program for review before the individuals perform any surgical procedures.

Definitions:

Major Survival Surgery – Any surgical procedure that penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection (such as laparotomy, thoracotomy, craniotomy, joint replacement and limb amputation).

Minor Survival Surgery – Any procedure that 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).

Non-survival Surgery – Any procedure where an animal is euthanized before recovery from anesthesia.

Aseptic Surgical Procedures – Surgery performed using procedures that limit microbial contamination so that significant infection or suppuration does not occur. Aseptic technique includes preparation of the patient, such as hair removal and disinfection of the operative site; preparation of the surgeon, such as the provision of decontaminated surgical attire, surgical scrub, and sterile surgical gloves; sterilization of instruments, supplies and implanted materials; and the use of operative techniques to reduce the likelihood of infection.

Sterilization - The process whereby all visible microorganisms are eliminated or destroyed. The criterion of sterilization is the failure of organisms to grow if a growth supporting medium is supplied.

Procedures for NON-Survival Rodent Surgeries

An animal is euthanized before recovery from anesthesia. At minimum the surgical site must be clipped, those participating in surgery should wear gloves, and the instruments and surrounding area should be clean.

Procedures for Rodent Major Survival Surgery

Pre-surgical Planning:

- Pre-surgical planning should include input from all members of the surgical team.
- The surgical plan should identify personnel, their roles and training needs; and equipment and supplies required for the procedures planned; the location and nature of the facilities in which the procedures will be conducted; and the perioperative animal health assessment and care. Most, if not all if this information is addressed in the UWM Animal Protocol Form.
- Pre-surgical planning should specify the requirements for post-surgical monitoring, care and recordkeeping, including the personnel who will perform these duties.

Surgical Facilities/Area:

- It is recommended that aseptic surgery be performed in dedicated facility spaces in either a dedicated operating room/suite or an area that provides separations from other activities within a laboratory or animal facility. If performing surgeries in lab areas is it important to note that the portion of the room may not be used for any other purpose during the time of surgery.
- Most bacteria are carried on airborne particles or fomites, so surgical facilities should be maintained and operated in a manner that ensures cleanliness and minimizes unnecessary traffic.
- All surgical areas must be clean, uncluttered, and sanitizable.
- Prior to an after completion of all surgical procedures, all organic debris should be removed from all work surfaces. The surfaces should be disinfected using solutions or other appropriate disinfectants. (Table1)

Surgical Instruments

- Surgical instruments, implants or supplies that come in contact with the animal must be sterilized for use in rodent survival surgery. (Table 2) Several techniques (steam, dry heat, ethylene oxide, or chemical agents) can be used for sterilization. Quaternary ammonium compounds and alcohol are not acceptable. Be sure to follow the guidelines for each method.

- For multiple surgeries, instruments should be disinfected between animals. After using a set of instruments, remove all organic material and then immerse the instruments in an appropriately diluted disinfectant. (Table 3) Be sure to follow the guideline for each method and rinse the instruments in sterile saline. One may also soak the instruments in 70% ethanol between animals. Instrument tips can be sterilized within a bead sterilizer, requiring 10-15 seconds within the heated beads. It is recommended that instruments must be allowed to cool for approximately 30-60 seconds prior to reuse.
- A new sterile instrument pack should be used after every 4 or 5 major surgical procedures.

Preparation of the Animal

- Prior to taking the animals to the surgery area, remove all hair for at least a centimeter on either side of the surgical site. Hair can be removed by clipping with a #40 clipper blade, shaving with a razor, plucking (in anesthetized mice or similar-sized rodents), or by using a depilatory cream. Then vacuum or otherwise remove loose hair.
- Clean and aseptically prepare the surgical site. Use an effective antiseptic surgical scrub solution (Nolvasan surgical scrub, Betadine Scrub, etc.). (Table 4) Carefully scrub the area with a new clean surgical sponge or sterile cotton swab. Scrub in a gradually enlarging circular pattern from the center of the incision site to the periphery. The sponge or swab should not be brought back from the contaminated periphery to the clean central area. Rinse the scrubbed area using 70% alcohol or sterile water. Repeat the scrub/rinse cycle for a total of 3 times. To prevent hypothermia, try not to wet the animal any more than necessary.
- Place lubricating ophthalmic ointment in the anesthetized animals' eyes to prevent drying.
- Prevention of hypothermia during surgery is important. Care should be given to keep the animal warm during a procedure that will last more than 30 minutes. Maintain body temperature using a circulating water blanket, warm water bottle, or equivalent external heat source taking care to not cause thermal burns to the animals' skin. The temperature of such devices should be set at 29-32° C (85-90° F).
- Once the animal has been positioned for the procedure one can spray or paint the surgical site with a compatible surgical scrub solution.
- Drapes should be used.

Preparation of the Surgeon

- Body covering such as a clean lab coat and sterile surgical gloves must be worn by all surgeons working in the immediate surgical field and anyone touching the animal's internal tissue.
- Surgeons should wear a clean scrub shirt or a lab coat. Surgeons should also wear a surgical mask.

- Surgeons should wash their hands with an appropriate surgical scrub (e.g. Betadine Scrub, Nolvasan Scrub). Surgeons should wear sterile gloves. Surgeons may wipe their gloves for 30 seconds with sterile gauze pads soaked in 70-90% alcohol between animals.

Operative Procedures and Intraoperative Monitoring

- The animal must be maintained in a surgical plane of anesthesia throughout the procedure. Analgesics must be given unless withholding them is scientifically justified and approved by the IACUC.
- Careful monitoring and timely attention to problems increase the likelihood of a successful surgical outcome.
- Monitoring includes routine examination of anesthetic depth and physiologic functions and conditions such as body temperature, cardiac and respiratory patterns.
- Recommended criteria that will be used to assess adequacy of anesthesia and animal intraoperative well-being include;
 1. Absence of response to toe and/or tail pinch
 2. Absence of spontaneous movement
 3. Respiration rate or pattern
- Begin the surgery with sterile materials and handle them aseptically. The sterile field must be kept sterile throughout the procedure. A sterile field must also be maintained for the sterile instruments, this can include the autoclaved instrument holder, a sterile drape, or the inside of sterile packaging. Sterile instruments and gloves must not touch anything outside the sterile field, otherwise they will be considered non-sterile.
- Close the surgical wounds using appropriate techniques and materials. (Table 5) Close the deeper tissue layers in one layer. A simple, continuous suture pattern using 3-0 or 4-0 synthetic absorbable suture is preferable. A simple interrupted pattern using natural absorbable (chromic gut) may also be used. Close the skin as a separate layer, using tissue adhesive, staples, wound clips or a 3-0 or 4-0 monofilament non-absorbable suture.

Postoperative Care

- Provide appropriate nursing support such as a quiet, darkened recovery or resting place, timely wound or bandage maintenance, increased ambient warmth and a soft resting surface, rehydration with oral or parenteral fluids and a return to normal feeding through the use of highly palatable foods or treats.
- After surgery, animals should be in a clean, dry and comfortable area where they can be observed frequently by trained staff. Prevent hypothermia by placing the animals in a warm room or cage. Animals may be placed inside their home cage on a paper towel. Heating pads may be placed under the home cage during recovery for proper thermoregulation. Supplemental heat can be provided beneath the animal's cage or by a heat bulb above the

cage. The cage should be warmed to no greater than 25° C (85° F). To prevent hyperthermia, animals must be provided a means to migrate away from the heat source once they are awake.

- Warmed (25-27 ° C) fluids administered during or after the surgical procedure can hasten recovery and reduce the risk of anesthesia induced hypothermia and dehydration. Warmed saline or balanced electrolyte solution given intra-peritoneally will speed recovery. Give .25-.5 ml to mice and 3-5ml to rats.
- Provide analgesics as appropriate. It is best to provide analgesia prior to surgery. Analgesics should be repeated as necessary as long as the animal is likely to show post-operative pain which is usually at least 48 hours.
- Return the animal to its routine housing only after it has fully recovered from anesthesia. They should be sternal and clearly beginning to wake up. To prevent cannibalism or suffocation, house rodents individually until they are ambulatory.
- After recovery from anesthesia, monitoring can be less intense but should include attention to basic biologic function (eating, drinking, urinating, defecating) and to behavioral signs of postoperative pain, monitoring of the surgical incision site for wounds that may break open along the surgical suture.
- Remove skin closures by 10 to 14 days post-operatively. Requests to leave skin closers in longer must be scientifically justified and approved by the IACUC.
- Members of the investigator's staff or other individuals to whom postoperative care has been delegated should oversee the animal daily until all sutures, wound clips or other implanted devices have been removed and keep the appropriate written records.

Records

- For reference there is an example Rodent Surgery Record posted on the Animal Care Program website that can be used by labs for surgical record keeping. A brief notation on the cage card should include the date of surgery and initials of the surgeon.
- These records must be readily available to the IACUC Committee or their designee, the attending veterinarian, and representative of regulatory and accrediting organizations. It is recommended that they be kept in the animal room or lab nearby.

Table 1. Recommended Hard Surface Disinfectants (e.g. table tops, equipment)

Always follow manufacturer's instructions for dilution 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.
Quaternary	Roccal ®, Quatricide ®	Rapidly inactivated by organic matter.

Ammonium		Compounds may support growth of gram negative bacteria.
Chlorine	Sodium hypochlorite (Clorox ® 10%) Chlorine dioxide (Clidox ®, Alcide ®, MB-10 ®)	Corrosive. Presence of organic matter reduces activity. Chlorine dioxide must be fresh; kills vegetative organisms within 3 minutes of contact.
Glutaraldehydes	Glutaraldehyde (Cidex ®, Cetylcide ®, Cide Wipes ®)	Rapidly disinfects surfaces.
Phenolics	Lysol ®, TBQ ®	Less affected by organic material than other disinfectants.
Chlorhexidin	Nolvasan ®, Hibiclens ®	Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses.
Hydrogen peroxide/peracetic acid/acetic acid	Spor Klenz	Contact time 10 minutes.

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

Table 2. Skin Disinfectants

Alternation disinfectants is more effective than using a single agent. For example, an iodophor scrub can be alternated three times with 70% alcohol, followed by a final soaking with a disinfectant solution. Alcohol, by itself, is not an adequate skin disinfectant. The evaporation of alcohol can induce hypothermia in small animals.

Agent	Examples*	Comments
Iodophors	Betadine ®, Propodyne ®, Wescodyne ®	Reduced activity in presence of organic matter. Wide range of microbicidal action. Works best in pH 6-7.
Chlorhexidine	Nolvasan ®, Hibiclens ®	Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses. Excellent for use on skin.

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

Table 3. Recommended Instrument Sterilants

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. <i>Only tips of instruments are sterilized with hot beads.</i>
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 ®, Cetylcode ®, 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.

Table 4. Recommended Instrument Disinfectants

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.
Chlorine**	Sodium hypochlorite (Clorox ®, 10% solution) Chlorine dioxide (Clidox ®, Alcide ®)	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. Rapid 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.

Table 5. Wound Closure Selection

Suture	Characteristics and Frequent Uses
Polyglactin 910 (Vicryl ®), Polyglycolic acid (Dexon ®)	Absorbable, 60-90 days. Ligate or suture tissues where an absorbable suture is desirable.
Polydioxanone (PDS ®) or, Polyglyconate (Maxon ®)	Absorbable; 6 months. Ligate or suture tissues especially where an absorbable suture and extended wound support is desirable.
Polypropylene	Nonabsorbable. Inert.

(Prolene ®)	
Nylon (Ethilon ®)	Nonabsorbable. Inert. General Closure
Silk	Nonabsorbable. (Caution: Tissue reactive and may wick microorganisms into the wound, <i>so silk is not recommended for skin closures</i>) Excellent handling. Preferred for cardiovascular procedures.
Chromic Gut	Absorbable. Versatile material.
Stainless Steel suture/wound Clips/Wound Staples	Nonabsorbable. Requires instrument for removal.
Cyanoacrylate (Vetbond ®, Nexaband ®, Tissue Mend ®)	Skin glue. For non-tension bearing wounds.

Suture gauge selection: Use the smallest gauge suture material that will perform adequately.

Cutting and reverse cutting needles: Provide edges that will cut through dense difficult to penetrate tissue, such as skin.

Non-cutting, taper point or round needles: Have no edges to cut through tissue; used primarily for suturing easily torn tissues such as peritoneum, intestine or blood vessels.

IACUC Guidelines on Survival Surgery for Aquatic Species

Survival Surgery:

The area in which the surgery will be conducted should be a dedicated space that is easily sanitized, uncluttered and away from the flow of traffic to minimize contamination from other laboratory activities. The work surface should be thoroughly cleaned and disinfected prior to and after each procedure. Surgical instruments must be sterilized prior to use. If repetitive surgery is being performed and only one set of instruments is available the instruments must be sterilized between animals. Disinfectants are not recommended as they may damage skin and cause an exuberant response of the mucus-producing glands. If disinfectants are used the instruments must be thoroughly rinsed prior to use.

Animals should be kept moist during surgery. If anesthesia is not maintained with the animal immersed or partially submerged in water, water should be dripped onto the skin or soft moist drape or gauze may be kept in contact with the skin. It is recommended to soak frogs in water for 1 hour prior to surgery. For procedures in which fish are operated on while out of water for lengthy procedures, delivery of oxygenated water to the fish must be maintained for respiration. Systems that deliver water to the mouth or gills also keep fish moist.

Post-Anesthetic Care:

Place fish/amphibians in un-medicated water in a holding tank for recovery. Closely monitor fish/amphibians recovering from anesthesia until they are swimming/moving normally and have completely regained their righting response. The animals should be monitored frequently for at

least 24 hours after recovery from anesthesia. For lengthy anesthetic periods, recovery may be prolonged.

Record Keeping (Surgical):

Describe and record associated clinical signs of affected fish and note disposition (treated, euthanized, submitted for diagnostic testing, etc.). Detailed records of individual animals should be kept for all animals undergoing surgical procedures and should include details of anesthetics (agent, dose, duration of event), procedures performed, monitoring of anesthetic depth during procedures and postoperative recovery and care. There is an example Aquatic Species Surgical Record on the Animal Care Program Website.