Regulations and Guidelines

According to the Animal Welfare Act (AWA), all survival surgery will be performed using surgical gloves, masks, sterile instruments and aseptic techniques. Not all survival surgeries on rodents require a dedicated facility, but must be performed using aseptic technique. The AWA 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. The 8th Edition of the Guide for the Care and Use of Laboratory Animals states: General principles of aseptic technique should be followed for all survival surgeries.

Justification for Applying Aseptic Technique in Rodent Surgery:

Literature contains numerous articles documenting subclinical infections that can become clinical diseases following stress or immune suppression. Historically, researchers have performed surgery in rodents non-aseptically. However, experimental evidence shows that infections take a subclinical profile in rats and mice. Improvement is seen in post-op recovery by increasing food/water consumption due to implementing aseptic surgical technique. Experimentally induced wound infections in rats were not associated with gross clinical or obvious behavioral signs. It is unsafe to assume there is anything special, in either way, about the resistance of rodents to infections. Rodent models have been used for antibacterial research in which rodents have been used to model human bacterial diseases, including surgery related conditions. This fact would suggest that there might be very little difference between rodents and other mammalian species, including humans, in the development of infections, including post-surgical infections.

Definitions

Asepsis: A condition in which living pathogenic organisms are absent; a state of sterility.
Aseptic surgery: The performance of an operation with sterile gloves, instruments, etc., and utilizing precautions against the introduction of infectious microorganisms from without.

Preoperative Preparation

Special Considerations:
Rats and mice have a high surface area to body volume ratios and rapid metabolism.
- With high metabolic rate and limited fat storage, energy depletion can be stressful.
- Pharmacological doses are higher than in larger species.
- Dehydrate faster per unit of time
- Rats and mice lose body heat rapidly through hairless areas. Hypothermia during surgery is a frequent cause of intra operative mortality.
- Higher cost in time- 1 hour surgery is equivalent to 4 hours in a cat.

Surgical Stress:

The major responses to surgery are characterized by an elevation in plasma concentrations of catecholamines, corticosterone, growth hormone, vasopressin, renin, aldosterone and prolactin, and by a reduction in plasma concentrations of FSH, LH and testosterone. Plasma insulin and glucagon concentrations fluctuate. The hormonal responses to tissue trauma produce varied increases in glycogenolysis, but after major surgery the response may persist for 4-6 hours. More prolonged changes in protein metabolism occur leading to negative nitrogen balance lasting for several days. Even minor surgical procedures can produce prolonged effects. Minimizing tissue trauma, preventing infections, controlling post-surgical pain and discomfort, and supporting the animal’s nutritional needs will reduce the magnitude of the metabolic response to surgery. The purpose of a survival surgical procedure is to produce an animal model that is defined and that has the smallest degree of non-treatment variability. An important objective is to return the animal to physiological normality, or to a defined state of abnormality, as rapidly as possible.
Preoperative Animal Preparations:
- Assess animal health status: FLSC recommends 1-2 weeks of acclimation to the animal facility to overcome transportation stress.
- Observe for clinical signs of disease: The animals should exhibit normal posture and movement, glossy coat, and bright eyes. There should be no sneezing, coughing or unusual respiratory sounds. The cardiovascular status of the animals should reveal bright pink coloration of ears and mucous membranes in albino animals. Normal intake of food and water should occur.
- Fasting is generally unnecessary. Because rats and mice do not vomit, they do not have the risk of intra/post-op vomition as in other species. If you will perform surgery on the gastrointestinal tract, you can fast the animals briefly up to a few hours. However, the reason for doing it should be considered carefully and weighed against the interruption of normal metabolic processes needed for homeostasis. For example, starvation will not empty the stomach unless it is for more than 24hr but it will seriously deplete glycogen reserves in the liver.
- Select the appropriate anesthesia. Injectable anesthetics are often used for short surgeries or ones that do not require deep muscle relaxation. They have the advantage of reduced instrumentation for the maintenance anesthesia. The drawbacks include less control over depth and recovery. The alternatives include inhalation anesthesia using a nose cone or endotracheal intubation. Inhalation anesthesia benefits include rapid induction and recovery and control over anesthetic depth. However inhalation anesthesia requires monitoring of the animal, investment in a specialized vaporizer and delivery system and anesthetic gas scavenging. For endotracheal intubation of rats use a 14-18 gauge venous catheter, trimmed to the length between the nose and thoracic inlet. See FLSC veterinary staff for guidance.

Surgical Preparation of the Animal:
- Support normal body temperature during anesthesia. Rats and mice have high surface area and lose body heat rapidly by conduction. A major cause of surgical mortality is hypothermia. Body temperature drops precipitously under sedation or anesthesia. Low body temperatures can cause irreversible shock and death. Place the animal on insulating materials and provide a heat source but avoid overheating. When using a heating pad, place the animal on towels or thick paper drapes as insulation between the animal and heating pad. When using a heat lamp, use distance to control the amount of heat that reaches the animals. Circulating hot water pads because of their safety in warming without danger of burns, are preferred. You can test the environment temperature by placing a simple thermometer in the vicinity of the animal for the approximate duration of a surgery (only 1-2 degrees higher than body temp is necessary). It’s easier to maintain normal body temperature that to reheat a chilled animal. If the animal is allowed to chill, there will be a reduction in circulation and organ function.
- Position animals carefully. Avoid placing excessive tension on limbs, which may cause neural damage, shut off circulation, traumatize joints or impair respiration. Use tape to secure the limb(s) in position. Remember limb movement is a good indicator of anesthetic depth.
- Surgical site preparation is essential to aseptic surgical technique. Clip fur along the incision site allowing ample margins to ensure fur does not contaminate the surgical field and area is sufficient for the surgical scrub of the incision line. Avoid removing too much fur to reduce the risk of hypothermia. Remove loose hair prior to the surgical scrub with either sticky tape or a vacuum.
- Carefully prepare the surgical site. A series of 3 surgical scrubs are performed using tamed iodine or chlorhexidine solution alternating with alcohol to remove debris. Alcohol is an ineffective antiseptic, and is used only to remove debris. Apply surgical scrubs with a swab or sponge (moist, not dripping) beginning with the incision line and moving toward the periphery. Never use a squeeze bottle or saturated sponge to apply solutions as these can wet the body inducing hypothermia.
- Preparation of the surgical field includes draping of the animal and is necessary when viscera or sterile instruments may come in contact with unprepared skin and fur. Surgical draping options include surgical paper drapes which can be cut and customized for each animal or surgery. They are inexpensive, disposable and autoclavable. Transparent, self-adhesive drapes can be used provided the animal’s body is dry.
- Never use the anesthetized animal’s body as a table. Do not rest your hands or instruments on the chest or abdomen. External pressure interferes with respiration and blood circulation.
**General Surgical Preparation**

Characteristics of common laboratory rodent surgery are smaller incision sites, fewer personnel in the surgical team, multiple animal (batch) surgeries at one sitting, and shorter procedure times as opposed to surgery in larger species.

**Site** - The elaborate operating suites mandated by the NRC Guide for larger species are not necessary for rats and mice. Necessary requirements for survival surgery in these species are a clean, neat, disinfected area dedicated to rodent surgery for the duration of the procedure and a separation of functions of animal prep, operating field and animal recovery. These may be adjoining areas on a long bench top. The rationale is to avoid contaminating the operating field with loose animal fur, splashes from incision site scrubbing, and bedding dust and fur from nearby cages.

**Instruments** - Surgical instruments must be autoclaved. If performing batch surgeries, and using the same instruments on multiple animals, wipe them clean with 70% alcohol and re-sterilize the instrument tips between animals preferably using a hot bead sterilizer. You may need two sets of instruments to alternate use between animals to allow the tips to cool before touching them to tissue. Chemical sterilization such as glutaraldehyde must be followed by a saline rinse. If you are doing a full day of batch surgeries, then use a fresh set of autoclaved instruments for the morning and the afternoon series.

**Surgeon** - Survival surgeries necessitate sterile surgical gloves be worn, not exam gloves. If performing batch surgeries, the gloved hands or fingers are rinsed with a chemical sterilant followed by a saline rinse. Sterilant residues on gloves will be irritating to tissues and will increase the risk for local infection. Hands should be dried with a sterile towel. Put on new sterile gloves if there are any tears or punctures. An assistant should be available to anesthetize and prep the rodents to minimize contamination of the surgeon.

**Maintaining Asepsis (ungowned)**
- Gloved hands should be held elevated above the waist and below the shoulders and should touch only the surgical incision, sterile objects within the surgical field.
- Once gloved, do not touch or lean over non-sterile areas. Do not drop your hands to your sides. Do not touch gloves to your skin or clothes.
- Do not drag instruments over the edges of the sterile field because they can become contaminated.
- Do not allow surgical instruments to fall below the edge of table. If an instrument does fall, the instrument is considered non-sterile and should not be picked up or reused until re-sterilized.
- Sterile surfaces are to be kept dry. Moisture can lead to contamination of the surgical area.

**Intra-operative Analgesia:**
Anesthesia is a state where all perceived sensations are absent. Because drug effects can vary, you must assess the depth of anesthesia prior to beginning a painful procedure such as surgery. The depth of anesthesia and the level of analgesia must be adequate to prevent the animal from feeling any pain in response to a surgical stimulus. Before making an incision, squeeze a paw firmly, but without injuring it, to test the animal’s perception or sensation of pain. If the animal withdraws its leg or if respiration rate increases, then the anesthesia is too light. Assess how much time elapsed from administering the anesthetic and compare that to the expected time of peak effect. You may have to wait longer for the anesthetic to take effect. Or, if surgery was delayed, the anesthetic may have worn off. If neither of these time factors may account for the inadequate anesthesia, it is possible that you may have to use a higher dose rate of your anesthetic.

**Preemptive Analgesia:**
Preemptive analgesia is the prevention of pain before it occurs. As an adjunct to general anesthesia, a local anesthetic is used to desensitize a body area before making an incision. This reduces the pain of the surgical wound postoperatively and in healing. Much of the post surgical pain is the result of the sensations produced in the skin and body wall of the incision area before making an incision. This reduces the pain of the surgical wound postoperatively and during healing. When skin and tissue are incised, local sensory nerves become excited and transmit impulses to the brain that are interpreted as pain. During general anesthesia, the animal is unconscious and is unable to perceive the neural stimulations from the incision site and so is unaware
of painful sensations. However, when the anesthetic has worn off, the brain will process these neural excitatory impulses, which continue postoperatively for days until the incision is healed. The result is that the surgical wound is painful and sensitive to touch and movement. If a local anesthetic is infiltrated prior to the incision, it will block the sensory neuroexcitation caused by cutting the tissues. When the animal wakes up there is a reduction in sensory stimuli from the incision area, and pain of the surgical wound will be greatly decreased both initially and throughout the period of wound repair. Inject a local anesthetic subcutaneously to infiltrate it in the vicinity where the incision will be made. Allow a few moments for it to diffuse and take effect before beginning the surgery. Lidocaine can be used, but it is short acting and will provide no lasting effects. Inquire about local anesthetics and post-op analgesics from the FLSC Veterinary Staff.

**Intra-operative Care**

During anesthesia and surgery, assess and provide support for body temperature, respiration and cardiovascular function. In rodents, elaborate instrumentation is not necessary. For monitoring respiration and circulatory function, you can periodically observe the animal’s appearance.

**Respiratory Problems:** The ears, muzzle, tail and any hairless areas will have a bluish tint if hypoxic. Evaluate the need for delivering oxygen. A tube delivering oxygen from a tank can be taped onto the table in the vicinity of the animal’s nose, a face mask may be made from a syringe case or syringe barrel or the rodent inhalation anesthesia machine with the nose cone in place can be run on oxygen only. Maintain airway patency by extending the animal’s head and neck in a straight line. Any blockages of the respiratory passages by blood, mucus, other material must be cleared. If respiration rate falls progressively (40-90 breaths/ minutes acceptable) reduce inhalation anesthesia or assist ventilation by gentle compression of the chest at a rate of 1 breath/ second if possible during a surgery. If surgery is complete, administer an anesthetic antagonist (if appropriate) or a respiratory stimulant.

**Cardiovascular function:** The ears, muzzle, tail and any hairless areas, normally pink, will blanch white if tissue perfusion is poor. Assess the cause of cardiac impairment.

**Anesthetic overdose:** Use an antagonist or an anticholinergic in the case of injectable anesthesia if appropriate.

**Hemorrhage:** Hemorrhage and loss of 10 % total blood volume is tolerable, but 20-25% loss will cause shock. Good surgical technique will minimize blood loss. Blood transfusion is ideal for inbred strains as cross-matching is not necessary. For surgeries with a high risk of hemorrhage a blood donor is recommended. Outbred strains generally have no problems when transfused once.

<table>
<thead>
<tr>
<th>Blood Volume</th>
<th>10% Blood Loss</th>
<th>20% Blood Loss (shock risk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse - 20 gram</td>
<td>1.5 ml</td>
<td>0.15 ml</td>
</tr>
<tr>
<td>Rat - 200 gram</td>
<td>15 ml</td>
<td>1.5 ml</td>
</tr>
</tbody>
</table>

**Fluid therapy:** Fluids support cardiovascular function and prevent dehydration. Animals will have reduced food and water intake for 1-2 days after surgery. Providing sterile, warmed, physiological fluids (SC, IP, PO or IV) can be used to compensate for hemorrhage and reduction in water intake postoperatively. Warmed fluids can be administered pre-operatively if a prolonged recovery is expected or extensive hemorrhage may be likely. Tail vein catheters may be placed pre-operatively to be available for IV infusions if necessary. Observe the animal for reduced water intake during the first 12-24 hours post-operatively and provide replacement fluids as indicated. The preferred route is intravenous (IV) but can be delivered according to the IACUC Fluid Administration Guidelines. If unexpected mortality occurs during the procedure, monitor heart rate and blood pressure in succeeding animals.

<table>
<thead>
<tr>
<th>Pre-operative Fluids</th>
<th>SC or IP fluids</th>
<th>IV fluids</th>
<th>PO fluids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse - 20 gram</td>
<td>0.14 ml</td>
<td>2 ml/100g/hr</td>
<td>1.2 cc /24 hr</td>
</tr>
<tr>
<td>Rat - 200 gram</td>
<td>1.4 ml</td>
<td>2 ml/100g/hr</td>
<td>12 cc /24 hr</td>
</tr>
</tbody>
</table>

**Surgical Technique**

Minimize contamination of the operative field during surgery by restricting the movement of gloved
hands and sterile instruments. Plan the incisions to avoid large vessels in the skin or body wall. Handle tissues gently and avoid excessive force in tissue retraction, which can cause necrosis. Avoid or minimize hemorrhage. If hemorrhage occurs, blot away blood with gauze sponge to remove a medium for bacterial growth. Never use a wiping action with gauze sponges, which will traumatize tissues causing renewed bleeding, instead use a blotting action. If a wound becomes contaminated, use warm, sterile Buffered Saline Solution to irrigate and cleanse the area.

**Needle Type:**

**Soft Tissues:** Use tapered (round-bodied) needles on internal tissues. This type of needle passes atraumatically through soft tissues and allows them to “seal” behind the needle. Avoid cutting needles in soft tissues to prevent tearing the tissue and to prevent cutting through blood vessels leading to more hemorrhage in vascular tissues (e.g. muscle).

**Skin:** Use a cutting needle in the skin. The dermis has tough fibrous tissue and cutting edges are needed to slide the needle through the skin. This minimizes trauma and irritation to the skin. As a result, the animal will be less likely to inflict self trauma to the sutured incision when a cutting needle is used. Avoid tugging and stretching the skin during closure as this increases soreness. Needles with swaged on suture impose less trauma on tissues than do threaded needles.

**Suture Material:**

Select suture material to be used based on tissue. For internal sutures an absorbable material is recommended unless permanent ligatures are needed. Absorbable material includes Vicryl, PDS, and gut. For skin layers use a non-absorbable mono-filament such as Prolene, nylon and polypropylene. Braided sutures, like silk, can wick bacteria into the skin and cause irritation, infection and increases the chances of animal self-trauma. Select the appropriate size suture for the tissue and the location of the wound. Large suture will be required for areas under tension; smaller suture is preferred in delicate tissues. Tissue Adhesives are acceptable for closing skin in rodents. Use adhesives sparingly to avoid glue residue on the skin as animals are likely to self-traumatize the area. Carefully place a tiny drop via an applicator tube onto the subcutaneous tissues and push the opposing edges of skin together, margin to margin. Excess glue can result in surgical gloves, instruments or gauze adhering to the wound. Avoid getting adhesive on the fur, or the animal may later open up the wound in the process of removing the glue from its fur. Apply adhesive drops 3-5 mm apart.

**Suture Layers and Patterns:** Generally, space sutures evenly with the suture penetrating the tissue ~ 5mm from the wound margin. Interrupted sutures and wound clips should be spaced ~ 5mm apart along the wound. Avoid drawing sutures too tightly as wound margins normally become moderately edematous. Tight sutures will strangulate tissue and become painful. When cutting suture ends avoid cutting so short that the knots unravel. In skin fold areas, suture strands may jab the skin and cause irritation especially with mono-filament nylon, because the cut end is stiff. Skin fold irritation may be avoided by altering the placement of the sutures, changing the length of suture strands.

1. **Body wall** (abdomen) - The suture line should be in a simple, interrupted pattern, using absorbable suture material. Do not use a continuous pattern. The body wall layer is an important one because it must take the tension in the body wall caused by animal movement. Animals do not restrict their mobility after surgery, and so this layer must be secure. If continuous sutures are used the danger of a broken suture or knot increases the possibility of incision dehiscence.

2. **Subcutaneous tissue** - The suture line should be in a single continuous pattern, using absorbable suture. This should be used in larger rats which have a sizeable amount of subcutaneous tissue; it is not used in mice. Closing this layer collapses the potential space between tissue layers, preventing seroma or abscess formation. The subcutaneous layer will not have the tension of the body wall, and so that suture strength is not needed. Therefore, the continuous pattern can be safely used for its advantage of speed in suturing.

3. **Skin** - The suture line should be a simple interrupted pattern, for the same reasons as for the body wall layer.

4. **Knot Tying** -
   - Tie all sutures (any layer) with square knots with a third throw.
   - Don’t cut knot strands too short to prevent unraveling of the knot.
   - If skin sutures are cut too long the animal may chew on them and in so doing, remove the suture.
• When applying autoclips avoid excessive pressure to prevent tissue ischemia.

5. Dehiscence – The splitting open of suture lines is referred to as dehiscence. There are multiple causes including suture strength failure, tearing of tissues due to tension, infection or seroma, or trauma to the suture line by the animal. Over-tight skin sutures are the most common cause of suture removal by animals. Over-tightening and low grade infection can cause dehiscence. Maintaining good aseptic technique prevents infection which can macerate wound margins and cause sutures to degrade and fall out.

6. Suture Removal - Suture or autoclips must be removed from the skin at 7-10 days post-surgery. The time will vary depending on the surgical site and rate of wound healing. Sutures or clips not removed can become embedded in the skin and cause irritation and possibly infection.

Postoperative Care

If possible, use anesthetic/sedative antagonists to recover the animal more quickly from injectable anesthesia. Yohimbine® is administered IV (preferred) or IP to reverse xylazine. To treat respiratory depression, Doxapram® at 5-10mg/kg IV or IP is used. Re-dose as necessary at 15 min intervals. Continue providing a source of heat until the animal is fully ambulatory. Provide clean bedding to avoid wound contamination. Assess food and water intake for several days, monitoring for dehydration. Animals may not drink for the first day post-op and fluid replacement is recommended. Follow the FLSC Rodent Dehydration Guidelines for assessment and treatment of dehydration.

Environment: Rats and mice prefer low lighting, quiet, and a place to hide. Provide enrichment tubes, Enviro-dry™ or other substrates for burrowing and hiding once recovered from the anesthetic. Also, provide clean bedding to reduce surgical wound contamination with feces and urine.

Pain Assessment: Observe animals for signs of pain or distress post-operatively. Most rodents are nocturnal and less active during the day, making it difficult to assess behavior at times of less than peak activity. Compare posture and activity with non-surgical animals. Rats and mice are able to mask pain, which is an evolutionary adaptation important for prey animals therefore pain assessment is more difficult. Most obvious signs will be a reduction in food and water consumption. If the procedure is likely to produce pain in humans, it should be assumed to be painful in animals and should be treated with analgesics. Signs that indicate pain or distress include a hunched posture, ruffled fur, red staining of eyes or nares, vocalization, greater or less tissue coloration, greater or less activity, objection to handling, reluctance to move, abnormal gait, and aggression. If pain or distress is observed or anticipated it is the investigator’s responsibility to provide analgesia. Post-op analgesia: The IACUC position on post-op analgesia and preemptive analgesics (which reduces pain postoperatively) requires provision for pain relief unless scientifically justified. Post-op analgesics fall into several categories; opioid and non-steroidal anti-inflammatory (NSAID). Choice is not limited to one or the other as both can be given and are additive in effect. Consult with the Attending Veterinarian for drug and dosage recommendations or refer to the IACUC Guidelines for Anesthesia and Analgesia of Laboratory Animals.

Record-Keeping

Record keeping for rodents is a regulatory requirement for AAALAC accreditation. Nonetheless it is a useful adjunct to research documentation for tracking changes in animal responses to anesthesia, surgical procedure and in recovery. Record data: animal ID, age, weight, sex, health status, anesthetic, duration of anesthesia, progress of surgery, perioperative care procedures, clinical observations of recovery, medications. The surgical record can assist in evaluating your surgical technique and point to potential improvements in technique by decreasing tissue trauma, improving aseptic technique, or suture technique or selection. It can be helpful to submit animals that have died for necropsy to determine if there are problems related to surgical infection or technique.

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