ADA Forsyth®

Animal Program Policy

Title: **Aseptic Surgery**
Date Created: 07/08/2011
Date Reviewed: 9/30/2026

Surgical procedures performed in animals at Forsyth are categorized as Major or Minor. In general, major surgery "penetrates and exposes a body cavity, produces substantial impairment of physical or physiological functions, or involves extensive tissue dissection or transaction," as stated in the 8th edition of the *Guide for the Care and Use of Laboratory Animals*. Minor surgery does not expose a body cavity and produces little or no physical impairment. All surgical procedures, whether major or minor, must be approved by the Forsyth IACUC, and must conform to the following guidelines. Aseptic technique is required for all surgical procedures.

1. Training

All individuals conducting surgery must undergo appropriate training, including training in the use of aseptic technique, which should be described in detail in the protocol form, subject to IACUC review. Prior to conducting surgery, all personnel must view video on the JOVE website, for information on patient and surgeon preparation. In addition, persons conducting surgery are required to undergo training (provided by the Director of Animal Care) in the use of the isofluorane apparatus if appropriate.

2. Pre-surgical planning

Persons planning surgery should consult with the veterinarian and/or the Director of Animal Care to develop a surgical plan. The plan should describe the equipment and supplies needed and detail the use of pre-and post-operative anesthesia and analgesia, and post-operative monitoring. A detailed plan should be assembled prior to protocol submission.

3. Facilities

Rodent surgery must be conducted in Procedure Room 1 (unless microbial infection is involved) in the animal facility. The surgery should be conducted in a dedicated area (for repeated procedures) or in an area that is carefully prepared and disinfected prior to surgery. If possible, surgery should be performed inside the Biosafety cabinet, although the limited space may preclude many procedures. Ensure that the surgical area is free of materials not directly involved in the surgical procedure. Prior to setting up the surgical field, clean the bench and nearby areas with Peroxigard or ethanol. Assemble all required items prior to initiating anesthesia. A post-operative recovery area should be prepared where animals can be kept warm after surgery.

4. Preparation of surgical instruments and equipment

Instruments should be autoclaved and sterile packs used within one month (sterility indicator, such as autoclave tape, should be used and the date should be written on autoclave tape). **Soaking instruments in alcohol and/or flaming is not acceptable.** A second set of sterile instruments should always be available as backup. When multiple surgeries are planned, the use of glass bead sterilizers (Steri 250, available in Procedure Room 1), or the use of additional packs are recommended. It is acceptable to operate on up to five rodents using one instrument set as long as blood and tissue debris are removed between procedures and sterility is not compromised. After these five animals, instruments must be re-sterilized using a hot bead sterilizer, or another sterile pack of instruments can be used. When using a hot bead sterilizer, sterile drape should be used to establish a sterile field on which instruments can be laid down to cool before tissue contact. Suture material and wound clips must be sterile and be removed from skin when healing is complete.

5. Preparation of the surgeon

Wash hands with Betadine,chlorhexidine, or chloroxylenol (GoJo). Dry with a sterile towel.
Sterile gloves and mask are required. When performing multiple surgeries on rodents in one session, new gloves should be used for each animal or gloved hands may be wiped with 70% alcohol and then dried with a sterile hand towel, but **only** if non-sterile items have not been touched. Non-sterile gloves should not contact the tips of surgery instruments. Sterile gloves, if reused, should be changed after every third surgery. Surgical hats and gowns are additional insurance against inadvertent contamination.

6. Preparation of surgical site and administration of pre-emptive analgesia

After anesthetic induction, administer first dose of analgesic.
Hair should be clipped away from the incision site with a size #40 clipper blade.

The size of the clipped area should be sufficient to ensure that:

- the surgeon has adequate visibility of structures deeper than skin
- the incision can be enlarged without compromising the sterile field if complications develop
- hair is not inadvertently incorporated in wound closure

The entire clipped area should be disinfected with a minimum of 3 cycles of scrubbing (in an outward centrifugal direction from the center of the incision site when possible) with a standard surgical scrub such as povidine or chlorhexidine soaps, followed by rinsing with 70% alcohol (sterile water or saline should be used for rinsing mucus membranes or open wounds). A final skin paint of povidine or chlorhexidine solution (not soap) is recommended. It is recommended that the surgical site should be draped with sterile disposable paper or sterile re-usable cloth drape sufficient to exclude surrounding hair from the incision and large enough to ensure a working sterile field around the subject.

7. Anesthetic regimens

Two anesthethic regimes are commonly used in rodents at Forsyth:

a. Ketamine 80-120 mg/kg and xylazine 5-10 mg/kg administered IP.
b. Inhaled isofluorane – 5% for induction, 1-2% for maintenance using the isofluorane vaporizer.

Other injectables and/or doses should be discussed with the veterinary staff during the planning process. For ketamine/xylazine, the xylazine reversal agent antisedan (atipamizole 1 mg/kg) should be available.

8. Pre-emptive analgesia

A first analgesic dose should be given right after induction of anesthesia. A common choice for rodents is Ethiqa (3.25 mg/kg for mice or 0.65 mg/kg for rats) . It may also be appropriate to apply perioperative local anesthesia such as bupivacaine or lidocaine. Use a 0.125% solution (you can buy 0.25% Marcaine and dilute it 50/50 with sterile saline) and a maximum dose of 2 mg/kg body weight. Before making the surgical incision, inject very small volumes (use a 25 gauge needle) at equidistant places approximately 0.5-1.0 cm apart, in an ellipse around the incision site. Wait 3-5 minutes before starting the incision.

9. Intraoperative monitoring and prevention of hypothermia

Respiratory rate and character should be monitored continuously. Evaluate pedal withdrawal reflex before starting surgery and when anesthetic depth is in question. Animals should be kept warm using a circulating water bath set to 37°C. Be careful during preparation of the surgical site to not get rodents excessively wet which promotes hypothermia

10. Post-operative care

Once the surgical procedure is completed, animals should be transferred to a clean empty cage lined with paper towels (not bedding). The cage should be placed half on-half off a heating pad set to medium or low to allow the animal to select the proper temperature. Once the animal is ambulatory and moving normally they should be transferred to a clean cage with clean bedding. Note that rats are particularly susceptible to pica (eating bedding) after opioid analgesia, they should be housed on paper bedding after surgery, and this should be monitored closely.

Upon return to the holding rooms, animals should be identified as being postoperative by appropriate post-op cards (available from Yoga). The use of unique cage cards is recommended, with spaces to make dated entries when you recheck animals or administer pre-op or post-op analgesics or fluids. These cards should remain on the cage until skin sutures or clips are removed and any post-op issues are resolved. If the surgery resulted in a clinically relevant abnormal appearance (such as a subcutaneous Alzet pump), details should be recorded on the regular cage card when the surgery card is removed. **Daily monitoring is required, and all monitoring steps recorded on the cage card must be dated and initialed.** Note that the analgesia regimen (drug name, dose, route and timing of administration) from the pre-operative period through the post-operative monitoring period (defined as when healing is complete – typically 7 to 14 days) must be performed on schedule as described in the approved protocol unless veterinary consultation changes the treatment plan. Separate surgical logs are required for major surgery and are strongly recommended for all surgeries. These forms are available in Procedure Room 1.

This document reflects the recommendation of the 8th edition of the Guide for the Care and Use of Laboratory Animals.