Common Surgical Procedures in Rodents

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Introduction

This chapter describes surgical procedures commonly performed in rodents in biomedical research. The previous chapter, Principles of Aseptic Rodent Survival Surgery, provided information necessary to performing surgery successfully, such as aseptic technique, pre-, peri-, and post-operative care concerns, and anesthesia. Therefore these details are not provided in this chapter, which focuses on the actual surgical procedures. Nonetheless, careful attention to sterility, thorough understanding of appropriate methods of anesthesia in rodents, and an understanding of concerns specific to care of rodents during and after surgery are imperative to the success of these techniques. It is also critical that the surgeons have experience in the use of surgical instruments and basic surgical technique. Practice with suturing techniques and anatomical dissections should be performed on inanimate objects and postmortem animals respectively prior to beginning experimentation on live animals. In most cases, the techniques in this chapter are described as they would be performed in a rat. When the differences in technique for mice are significant, such as catheter sizes, these are discussed within the relevant section. Surgical procedures are listed in alphabetical order. The final section focuses on special considerations for surgery in generation of genetically engineered mice. This section was contributed by Patricia A. Brown and Shelley Hoogstraten-Miller.

Adrenalectomy

The adrenal glands are small, pink organs located near the anterior pole of each kidney. The anesthetized rat should be placed in ventral recumbency and an area on the mid-dorsum (thoracolumbar junction) shaved and prepared for aseptic surgery. A midline incision 1 - 2 cm long is made just caudal to the peak of the animal’s dorsal hump (Fig. 1). Hemostats or blunt-tipped scissors are inserted subcutaneously through the incision and used to bluntly dissect the connective tissue down both sides a short distance (about 1/3 of the distance down the lateral abdominal wall). The skin incision is then pulled laterally to one side to expose the muscle just caudal to the last rib and a small incision made in the muscle to enter the peritoneal cavity; this incision is just large enough to retrieve the adrenal gland back through the incision. On the left side, the spleen should be visible directly underneath or slightly to the left of the incision (Fig. 2). Curved forceps are inserted through the incision into the peritoneal cavity and used to hold the spleen laterally. A second pair of forceps is used to move the incision over the gland, which is usually surrounded by adipose tissue just in front of the kidney. Once the gland is located, it is manipulated by grasping periadrenal fat and exteriorized (Fig. 3). This may require freeing up some of the fascial connections between the kidney and adrenal gland. Care should be taken not to grasp the gland itself because tearing of the gland may result in leaving functional residual tissue within the abdomen. Clamp the vessels at the base of the adrenal gland with both forceps. The forceps are then used to tear away the gland and its surrounding tissue. The tissue stump is then returned to the abdomen. No hemostasis is necessary as bleeding should be minimal. On the right side, an incision is made in the abdominal muscle similar to the left side, but the liver must be moved cranially to view and retrieve the adrenal gland. Closure of the abdominal muscle may be necessary if the incision is large (greater than 3 or 4 mm), such as in larger rats. If needed, one should use a single interrupted suture with 4-0 absorbable suture. The skin incision should be closed with either a wound clip or using 1-2 simple interrupted sutures with a non-absorbable suture.
Figure 1. Location of incision for adrenalectomy on dorsum of a rat

Figure 2. A large incision is present for demonstration purposes to illustrate the location of the left adrenal gland in relation to the spleen and kidney. The arrow points to the adrenal gland nestled in adipose tissue just cranial to the anterior pole of the kidney.
Post-operatively, replacement of mineralocorticoids and corticosteroid is necessary to maintain homeostasis. A standard 0.9% saline or a saline/dextrose solution (0.9% saline and 10 g/L dextrose) can be administered instead of drinking water. Corticosteroid replacement can be achieved with hydrocortisone acetate (0.1 mg twice daily by subcutaneous injection in rats).

**Bile Duct Catheterization**

The bile duct runs from the hilum of the liver through the pancreatic tissue to the duodenum, where it is surrounded by the muscular sphincter of Oddi. The duct is approximately 1 mm wide in the rat and moderately translucent. There is no gall bladder in the rat. Substances excreted by the liver into the bile duct may be reabsorbed in the gut, and carried back to the liver by the portal vein as part of the enterohepatic circulation. A dissecting microscope can be useful for successful catheterization of the bile duct.

The anesthetized rat is placed in dorsal recumbency, prepared for aseptic surgery, and a midline abdominal incision is made starting at the xiphoid cartilage and extending caudally about halfway down the abdomen (approximately 4 cm long in a rat). A technique, along with excellent diagrams, is described in Waynforth et al. [1]. This technique involves arching the back up using rolled gauze, to facilitate visualization of the bile duct. For exteriorization of the catheter and chronic access, a needle with an interior diameter just large enough to slide the catheter through is passed through the skin at the side and towards the back of the rat, and into the abdominal cavity taking care not to puncture the gut. The needle is used to exteriorize the catheter.

The duodenum and a small part of the intestine is pulled out and placed on a saline-moistened gauze pad. The course of the bile duct through the pancreatic tissue should now be visible (Fig. 4). The
major lobes of the liver must be moved cranially against the diaphragm with a retractor or with a moistened gauze pad. One can identify the portion of the bile duct near the hilum of the liver. In order to collect pure bile without contamination of pancreatic juices, the duct should be catheterized near the hilum. Forceps are used to carefully clear the bile duct of surrounding connective tissue for a distance of approximately 1 cm. The surgeon can then pass a doubled-up length of suture under the bile duct and cut to create two separate threads. One suture is tied to create a ligature just cranial to the pancreatic tissue to obstruct bile flow. A single hitch should be thrown with the second suture strand about 0.5 cm cranial to the first ligature, closer to the liver (Fig. 4).

![Figure 4. Bile duct near the hilum of the liver, with ligatures pre-placed prior to cannulation.](image)

A polyethylene (PE-10) or silicone catheter, 0.28 mm inside diameter (ID) and 0.61 mm outside diameter (OD), is cut so that a short beveled point is obtained. The bile duct near to the first ligature is held with fine forceps and partially transected with micro-scissors or punctured with a 23 gauge needle. The catheter is introduced into the duct towards the liver and advanced until several mm past the cranial suture. This suture is tightened and the knot completed to secure the catheter in the bile duct. One can then tie another throw around the catheter with the caudal suture. Bile should be visible within the lumen of the catheter.

The catheter is then exteriorized by passing it through the end of the needle and passing the needle back out of the animal’s skin, bringing the bile duct catheter with it. The catheter should lie in the
abdominal cavity with some free play to allow free body movement once the animal regains consciousness, and without twisting or kinking. The liver and intestines are then returned to the abdomen. The flow of bile in the catheter is confirmed before suturing the catheter to the skin. The catheter can be secured to the skin by use of tape placed around the catheter, which is then sutured to the skin using simple interrupted sutures, or by use of a silicone disc/button which is slid over the catheter and sutured to the underside of the skin. The abdominal incision is closed in routine fashion.

If the catheter is to be used for acute studies only, then the rat can be restrained in a "Bollman-type" restrainer or similar device during the collection period when the animal has recovered from anesthesia. For chronic collection studies, the catheter will need to be protected, either by use of a tethering device and harness or by attachment of the catheter to a vascular access port and placement of the port in a subcutaneous pocket on the dorsum. The difficulty with chronic collection studies is that gravity significantly aids in flow, and the collection system must take this into consideration.

**Cesarean Rederivation**

Cesarean rederivation is often used to obtain pathogen-free mouse or rat pups from a mother that is either infected with a known pathogenic agent or is of unknown health status. In this situation, extra precautions must be taken to ensure that cross-contamination does not occur. These include dipping the mother in disinfectant, such as dilute iodine, immediately after euthanasia, and using a second set of sterile instruments for opening the uterus from those used to open the skin. If survival of the dam is not necessary, it is best to euthanize it by cervical dislocation without any anesthesia to avoid cardiovascular and respiratory depression of the pups. This procedure should be performed as close as possible to the expected time of parturition to increase the likelihood of producing viable pups. For mice, this should be performed on Day 19 or Day 20 (with day of vaginal plug observation being Day 0), depending on the strain. For rats, Day 20 is typically used. It is also important to have suitable foster mothers available. The foster mothers should:

- Ideally be of a stock or strain that typically has strong maternal instincts. Most outbred stocks do well. With regard to inbred strains, BALB/c mice are typically good mothers.
- Foster mothers should have newborn litters preferably no more than 2 - 3 days old at the time of rederivation.
- Using a foster mother with pups of different coat color from those pups being rederived will allow the two sets of pups to be easily distinguished once they are mixed together.

The euthanized or anesthetized pregnant animal is placed in dorsal recumbency on a sterile surface, and a long ventral midline incision made from the xiphoid process to the pubis. Care should be taken when opening the abdominal wall to not accidentally enter one of the uterine horns, as these are often lying immediately dorsal to the abdominal musculature. One horn should be exteriorized and placed on gauze soaked with warm saline. The horn is carefully opened along its entire length using scissors, on the side opposite the placental discs. Working with one pup at a time, forceps should be used to ligate the umbilical blood vessels between the placental disc and the mother’s uterus. The fetus is then pulled away with its amniotic sac and placenta still attached. The pups should be handed to an assistant who can then remove the amniotic sacs and provide postpartum care to the pups. After all pups are removed from one horn, the forceps can be removed. If the blood vessels are large (e.g., in rats in advanced pregnancy) they should be ligated prior to removal of forceps; otherwise, gentle pressure with gauze or a cotton-tipped applicator will provide sufficient hemostasis. Once the pups have been removed from one horn, one should quickly proceed to the other horn and repeat the procedure for removing the pups. If the dam is intended to survive from this procedure, the abdominal muscle can be closed in standard fashion with simple interrupted
sutures using an absorbable suture, and the skin closed with non-absorbable suture or staples. An audiovisual step-by-step instructional guideline of this procedure in mice is available online. For non-survival procedures, an alternative method is to make a cut across the caudal vagina and dissect out the entire uterus from the mesentery. The entire uterus is then placed in a dish with disinfectant, then removed onto a sterile absorbent surface for removal of the fetuses.

The pups should be gently dried and stimulated until they are breathing well and gain a healthy pink color indicating good tissue oxygenation. Gently squeezing the tail or a paw, or providing oral stimulation using a cotton tipped applicator should evoke a response from a healthy full-term pup. The pups should be transferred to the foster mother as soon as possible. Removal of the natural litter from the foster mother shortly before the procedure, and gently rubbing the two litters together along with some of the nesting material from the home cage should provide sufficient olfactory masking of the new pups to avoid rejection. Depending on the total number of pups in the two litters, some of the natural pups may need to be removed. A total of 8 to 12 pups will stimulate good milk production without overburdening the mother.

Jugular and Carotid Catheterization

Vascular access is often needed to either infuse a substance, obtain repeated blood samples, or to monitor blood pressure. This may be done as an acute procedure where the experiment takes place over the period of one or several hours and the animal is euthanized while under anesthesia, or as chronic cannulation to allow for vascular access over a prolonged period of time (days to weeks). Chronic catheterization requires that utmost attention be paid to sterility and the prevention of thrombus formation which obstructs cannula flow. Infections are the leading cause of early failure for indwelling catheters. The type of catheter used also has significant implications on biocompatibility and long-term patency. The most common materials used are polyurethane and silicone. Polyurethane or polyethylene both have good rigidity which makes vessel entry easier, but may induce more inflammatory response from the luminal surface. Silicone is more flexible and has been shown to be more resistant to infection and cause less tissue trauma within the vessel but is harder to work with. Newer catheters, such as Renathane™ (Braintree Scientific Inc. Braintree, MA, USA), Hydrocath™ (BD Medical, Franklin Lakes, NJ, USA) and CBAS™-coated catheters (Carmeda AB, Stockholm, Sweden) have anti-thrombotic coatings and seem to also resist infections better than standard non-coated catheters. Use of retention beads is also recommended. These serve as anchoring points for the catheter in the tissue, and can be "home-made" using Silastic glue, or purchased with the catheter as either movable or fixed rings on the catheter. There are numerous references available addressing catheter properties, effective flushing solutions, and ways to minimize the complications of infection and catheter obstruction, so this topic will not be addressed in any detail here. For all vascular catheterizations, the catheter should be pre-filled with heparinized saline solution (50 IU/mL), and is usually attached via a 23G needle to a syringe similarly filled with no air bubbles. For rat jugular veins, a 3Fr (OD 0.94 mm; ID 0.51 mm) catheter works well. In mice, catheters are either tapered to a small diameter or the smaller intravascular portion (1.2Fr; OD 0.41 mm, ID 0.23 mm) of the catheter is inserted into a larger diameter tubing (3Fr) for the extravascular portion.

Venous access can be achieved via the jugular vein, femoral vein, or tail vein. Similarly, arterial access can be obtained by catheterizing either the carotid or femoral artery. The techniques are largely similar and differ primarily in approach and method of securing the catheter outside the animal.

Catheterization of the Rat Jugular Vein
The anesthetized rat is placed in dorsal recumbency with its head towards the surgeon. The right external jugular vein is most often used as it feeds directly into the right atrium. The tip of the catheter should ultimately lie at the junction of the precava and right atrium. An incision approximately 1.5 cm long is made in the skin on the right ventrolateral aspect of the neck above the clavicle. In mice, the procedure at this point may be more easily performed using a dissecting microscope or loupes. The right external jugular vein is dissected free of surrounding fascia with the caudal most landmark being where the vein courses underneath the pectoral muscle (Fig. 5). The bifurcation into the maxillary vein and the linguofacial vein may be visible rostrally. Once there is a cleared section of vein (5 - 10 mm) it can be stabilized by passing two loops of 4-0 silk suture underneath the vein. This is most simply done by passing a small forceps underneath the vein, grasping a doubled piece of suture in the teeth of the forceps, then drawing the suture back underneath with the forceps. The suture is then cut into two pieces, one of which is moved to the most cranial (anterior ligature) aspect and the other moved caudally (posterior ligature). After filling that portion of the vein with blood, the anterior ligature is tied tightly to occlude blood flow returning to the heart. The posterior ligature is tied with a single loose half-hitch and can be used to create tension on the vessel during venotomy and catheter insertion (Fig. 6). Some surgeons like to place a flat instrument or metal strip underneath the vein during venotomy to provide stabilization and a rigid surface against which to catheterize. A venotomy is made posterior to the anterior ligature, using either fine iris scissors, micro-scissors, or a needle tip. The venotomy can be widened by inserting the tips of Dumont #5 forceps and spreading gently.

Figure 5. Jugular vein visible after blunt dissection of surrounding tissue.
Angled Dumont forceps can be used to keep the venotomy site open while the catheter tip is introduced and advanced towards the heart using a fine pair of forceps to grasp the catheter and push it gently forward. A commercial catheter introducer can also be used for this procedure. The distance that the catheter must be advanced to place the tip in the right atrium will depend on the size of the animal and should be pre-determined in a pilot study. For infusion studies, a shorter distance of insertion is adequate, as long as good blood flow is obtained at the site of placement. Typically, for a rat weighing 250 - 300 g, a distance of 32 mm (starting 3 mm cranial to the pectoralis major muscle) will place the tip at the entrance to the right atrium. In mice, the catheter is advanced approximately 10 mm. In rats, the first retention bead may be inserted within the jugular vein just proximal to the venotomy site. If blood can be easily withdrawn from the catheter in this position, the posterior ligature should now be tied securely around the vein and catheter, and distal (anterior) to the retention bead which is located inside the vein. The anterior ligature, already used to ligate the vein, should now be tied around the catheter cranial to the second (distal) retention bead (Fig. 7). In mice, the more proximal bead should lie between the two ligatures as with the rat, but will sit over the vein rather than inside the lumen. At this point, the catheter should be flushed with a "heparin-lock" solution (100 units heparin/ml saline) and plugged at its end with a sterilized pin, or clamped with a small vascular clamp. The pin is typically stainless steel and made by removing an appropriate size hypodermic needle from its hub and filling it with silicone rubber cement, or by using an appropriate size diameter piece of stainless steel wire. Alternatively, if the experiment will begin immediately the catheter may be attached directly to a pre-filled syringe and needle.
To exteriorize the catheter it will need to be tunneled through the subcutaneous tissue to exit at a site on the dorsum between the scapular blades. This surgical site should be prepared in advance at the same time as the neck incisional area is prepared and protected (kept sterile) during the initial catheterization procedure. At this point, a small stab incision is made on the dorsum and a pair of curved forceps or a trocar is passed under the skin towards the site of the venous catheterization. The catheter is grasped by the forceps or passed through the trocar and brought back out the dorsal incision. Some slack should be kept in the catheter where it makes its turn in the neck area. This can be accomplished by placing a suture around the catheter and fascia and muscle in the neck region just after its turn from the vein towards the dorsum, or a small loop can be made with the catheter which is then sutured to the neck musculature. This suture should be tied slightly loose to avoid constricting or kinking the catheter at this point. The extra slack in the catheter will help prevent tension from being transferred directly from the manipulation site of the catheter on the dorsum to its position in the jugular vein. After exiting the dorsal incision, the catheter will either be cut to a short length (2 - 3 cm) and plugged, for intermittent access; or attached to a tethering system, or to a vascular access port secured in the same location on the dorsum for more frequent access. Both skin incisions should now be closed. A video film of chronic catheterization of the jugular vein in the rat can be purchased through the Norina\textsuperscript{tm} database. Alternative methods of securing the cannula to the crown of the head are described in the literature.

**Ovariectomy**

The mid-dorsal thoracolumbar region is clipped to remove hair and prepped for aseptic surgery. A single mid-dorsal incision in the skin is made, farther caudal from the hump of the back than the incision used for adrenalectomies. This incision only needs to be about 5 mm long once proficiency in the surgery is gained. Following skin incision, a second incision is made about halfway down the side of the abdominal wall into the dorsolateral musculature to enter the abdominal cavity in the region of a periovarian fat pad. In mice, this fat pad is usually visible through the abdominal wall.
and the incision can be made directly over the fat pad. The ovary is exteriorized by grasping peri-ovarian fat with tissue forceps, and the pedicle ligated and excised between the uterine horn and the fallopian tube (Fig. 8). In mice and small rats, ligation is unnecessary as bleeding is minimal. The process is repeated for the other side. Both incisions in the abdominal musculature are closed with 4-0 absorbable suture. The skin incision is closed with a wound clip, surgical glue or suture.

Figure 8. Expanded incision to illustrate anatomy of uterine horn, ovary, and Fallopian tube. The ovary is removed by excising between the uterine horn and the Fallopian tube. Note that in surgical procedures, the skin and muscular incisions should be smaller than demonstrated here.

**Pinealectomy**

The pineal gland is a very small (1 - 2 mm), round, translucent organ located on top of the brain. It functions to secrete melatonin and may help regulate the pituitary. Surgical removal of this gland (pinealectomy) generally requires placement of the animal’s head in a stereotaxic frame. By using the incisor nose clamp and temporal bone cups or ear bars the skull position is stabilized. Coordinate measurements are not necessary for this procedure as major vessels on the surface of the brain serve as accurate landmarks. However, for most cranial surgeries, verticality and flatness of the skull must be carefully verified so that coordinates are accurate. Once the animal is placed in the stereotaxic device (ventral recumbency with incisor clamp and ear bars in place), ophthalmic lubricating ointment should be applied to the eyes to prevent corneal damage during surgery. If the animal is lying on a metal surface, it is important to wrap the body in insulating material (paper or cloth drape, bubble wrap, thin styrofoam) to help prevent hypothermia.

The surgical site is aseptically prepared as with other surgical locations. Use of a long-lasting local anesthetic such as 0.25% bupivacaine provides analgesia during and after the surgery. The skin is cut longitudinally on the midline about 1.5 cm long. The periosteum overlying the skull is then scraped off of the bone using a dental scraper or a blunt probe. Bone wax is used as needed to control bleeding from the skull. The skin should be retracted and a small hole drilled using a dental drill above the right cerebral hemisphere near the junction of the superior sagittal sinus and the transverse sinus (Fig. 9). Some methods describe removing a rectangular or circular section of skull in this location. A small cut is made in the dura mater with microscissors. The pineal gland should
be visible underneath the opened dura, and can be retracted with forceps, and excised using blunt
tipped, curved iris scissors. It may be necessary to retract or to ligate and resect the superior saggital
vein to adequately expose the pineal gland. The skin incision is closed in standard fashion using
non-absorbable suture.

Figure 9. Diagram of skull sutures demonstrating intersection of transverse and sagittal sutures for
access to the pineal gland. To view click on figure

**Portal Vein Catheterization in Rats**

There are many references describing portal vein catheterization and the reader should familiarize
him/herself with some of these. Silastic tubing is most often used, typically 0.02-inch ID (0.5 mm)
and beveled at the distal end to aid in insertion into the vein. A ring of silicon glue or retention bead
should be placed 1 cm from the tip to provide an anchoring point for suturing. For chronic
catheterization, a second bead should be placed 4 cm from the first one to be used to anchor the
catheter to the abdominal wall as described in Strubbe et al.. The catheter should be sterilized prior
to surgery if the rat is recovering from surgery. Figure 10 diagrams the anatomic location of the
portal vein and its derivation.
Figure 10. Diagrammatic representation of portal vein anatomy.

The animal is placed in dorsal recumbency and the ventral abdomen prepared for aseptic surgery. A midline incision approximately 5 cm long is made in the skin starting 2 - 3 cm caudal to the xiphoid process. A similar incision is made in the abdominal musculature. The duodenum is reflected laterally to the right to expose the portal vein (Fig. 11). The area of the vein between the splenic and the gastroduodenal veins (still considered the superior mesenteric vein here) is cleared of any attached tissue and clamped using small vascular clamps. A 7-0 silk suture is passed through the superficial wall of the vein close to the distal clamp (farthest from liver) and a single throw formed loosely. The vein is then punctured using a 20 gauge needle just proximal to this suture. The cannula is inserted through this hole, the downstream clamp is removed and the cannula advanced into the portal vein up to the silicon bead. The bead is secured in place by tightening the knot in the suture and finished with a ligature around the catheter behind the bead. The remaining clamp is removed as quickly as possible to restore full blood flow and the organs returned to the abdomen. For acute procedures, it is possible to secure the cannula to the superior mesenteric vein by using HistoAcryl™ glue (Braun Melsungen, Germany) instead of using silicon beads, along with a ligature using 6-0 or 7-0 silk around the vein.
Figure 11. Asterisk identifies the portal vein as it courses cranially towards the liver.

For chronic catheterization, the second silicon bead is secured to the internal abdominal muscle near the xiphoid cartilage in a location that allows free movement of abdominal organs without any tension on the catheter, but also attempting to minimize risk of bowel entrapment. The cannula is tunneled subcutaneously to exit either on the dorsum between the scapulae or on the crown of the head where it is secured to the skull with hardware (see Strubbe et al., for details). The cannula should be filled with a heparin solution (50 IU/ml). The abdominal and dorsal incisions should be closed in standard fashion. For long-term maintenance the saline should be replaced with a 60% polyvinylpyrolidone (PVP, Irvine Scientific, Santa Ana, CA, USA) solution in saline with 500 IU/ml heparin or dextrose 50% / 50% heparinized saline solution.

A video film describing this technique in rats is available for purchase through the Norina™ database oslovet.veths.no/NORINA/search.html.

**Skin Grafting**

This procedure is used to evaluate host vs. graft response in immunologic experiments. The successful transplantation and acceptance of a skin allograft indicates immune tolerance. Although
the surgical procedure itself is straightforward, postoperative maintenance of the graft through the healing period can be difficult as the graft is fragile and subject to disruption by the host animal.

The donor skin is usually taken from the lateral thoracic wall. An anesthetized rat or mouse is positioned in lateral recumbency with the four limbs taped or secured away from the trunk. The hair over the lateral thorax is removed with clippers and the skin prepped with antiseptic solution. Use of a "plasticizer" solution to remove remaining loose hair has been described. The skin should be grasped with forceps and a full-thickness graft obtained by cutting a circle of skin approximately 1 inch in diameter. The graft should be placed hair side down onto saline-moistened filter paper in a Petri dish or other sterile shallow container. The muscle and connective tissue underlying the epidermis are then removed by scraping the underside with the edge of a scalpel blade. Once the muscle layer (panniculus carnosus) is completely removed, the graft should be placed on clean, moist filter paper with the hair side facing up. The graft should be covered with the Petri dish lid and kept moist until needed.

The recipient rat or mouse should be prepared as soon as possible after obtaining the donor graft. The recipient (host) is positioned in similar fashion as the donor animal was in lateral recumbency with the lateral thoracic area prepped for aseptic surgery. A portion of the skin on the lateral thorax is grasped with forceps and tented up away from the body. The skin at the point of maximal tension is then incised with sharp curved scissors. This creates a skin defect without cutting and removing the underlying panniculus carnosus and leaves a vascular bed intact for receipt of the graft. The skin is then incised further to create a circular defect similar in size to the donor graft. Active bleeding in the bed should be controlled using pressure and dry gauze. The skin graft is then placed in the bed and if necessary trimmed to fit neatly within the bed. If the donor and recipient hair colors are identical, the graft should be placed so that the direction of hair growth is opposite from that of the host animal. Some surgeons prefer to suture the graft in place by placing sutures at opposite ends of the circle using 6-0 or 7-0 chromic gut on a small taper needle. A maximum of 4 sutures equidistant from each other should be used. If a rectangular graft is used, a suture should be placed at each corner. The graft is covered with a gauze pad and pressure applied to hold the graft in place. A sterile nonadherent pad is then placed over the graft, taking care to not dislodge the graft during placement. The pad is bandaged in place using roll gauze and adhesive bandage or Paris plaster. This bandage should remain in place for 9 - 10 days to allow full healing of the graft. A photograph of a white A/J mouse carrying a skin graft from a CBA mouse is shown in Figure 12.
Figure 12. Skin graft from a CBA mouse transplanted and fully accepted into an A/J mouse (photo courtesy of Leslie Brent)

**Vasectomy or Orchiectomy**

Mice are often vasectomized to be mated to females used for embryo transfer. The female becomes pseudopregnant, her body geared for a pregnancy because she has been mated. Males used for this purpose are vasectomized at 6 - 8 weeks of age. Following vasectomy, the mice are allowed to recover for two weeks before being test-bred to confirm their sterility. Access to the testicles can be either through the scrotum or through a laparotomy.

**Scrotal Approach**

A small incision is made at the tip of the scrotum. The tunic is opened and the testis, cauda epididymis, vas deferens, and the spermatic blood vessels are exteriorized. For an orchiectomy, the blood vessels and vas deferens are then ligated with 4-0 absorbable suture or alternatively can be cauterized. The testis and epididymis are then removed. The remaining tissue is returned into the sac and the procedure repeated for the other testis. If a vasectomy is needed, only the vas deferens is ligated with two sutures placed 3 - 5 mm apart. The vas deferens is then cut between the two ligatures. Some surgeons prefer to actually remove a piece of vas deferens between the 2 ligatures and keep both pieces until finished to verify that both sides were done. The skin incision can be closed with a wound clip, nonabsorbable suture or surgical glue. If suture is used, animals must be monitored carefully as chewing sutures is a frequent complication.

**Abdominal Approach**

Using a pair of curved forceps and small scissors, a midline skin incision is made along the lower abdomen approximately 0.5 inch anterior to the genitals, and about 1.0 cm long. The body wall is incised and the bladder located. Both tubes of the vas deferens will then be visible lying on either side. The left vas deferens is gently grasped with forceps and a section lifted clear of the incision (Fig. 13). The vas deferens is then ligated and sectioned as described above. When both sides have been done, the incision in the body wall is closed with 2 - 3 interrupted sutures and the skin closed in standard fashion.

Figure 13. Vasectomy in a mouse. Vas deferens and testis labeled.
Special Considerations for Embryo Implantation Surgery in Mice

This section discusses the principles of aseptic surgery as applied to one of the most common rodent surgical procedures, embryo implantation into a recipient mouse. This example is not the only way to perform surgery aseptically. However, when considering alternative practices, the following points should be followed:

- The animal must be maintained in a surgical plane of anesthesia throughout the procedure.
- Begin surgery with sterile instruments and handle them aseptically.
- Best practice dictates always using sterile instruments and donning new surgical gloves between each animal. However, instruments and gloves may be used for a series of similar surgeries provided they are maintained clean and disinfected between animals.
- Provide analgesics as appropriate.

When performing surgery in mice, many choices for anesthesia, both injectable and inhalant, exist. Frequently, transgenic surgeries require multiple work stations and multiple animals anesthetized at any given time. Inhalant anesthesia systems are available allowing multiple workstations from one system. They provide a safe, consistent level of anesthetic depth with rapid induction and recovery times. Their disadvantage is the initial expense, space needed for the equipment and need for a scavenging system. Injectable anesthetics are readily available and generally reliable once personnel are trained in their use and familiar with dosages. One of the most common injectable anesthetics used for transgenic surgeries is tribromoethanol (formerly available under the trade name Avertin). Injectable anesthetics other than tribromoethanol, such as a ketamine-xylazine mixture, or inhalant anesthetics are also acceptable and may be considered preferable at some institutions. Tribromoethanol has been associated with adverse effects including peritonitis, fibrous adhesions in the abdominal cavity, and decomposition to toxic components. A veterinarian should be consulted for advice on selection and administration of anesthesia. Anesthetic agents selected must be approved in the IACUC protocol.

Pharmaceutical grade tribromoethanol is not commercially available in the United States, requiring it to be prepared in the laboratory. The solution must be prepared aseptically and filtered through a 0.2 μm filter. This will remove debris, most bacteria, and some viruses. The anesthetic solution should be stored at 4°C in a sterile, amber or aluminum foil-wrapped bottle. When diluting the stock solution of tribromoethanol to the working solution, it is important to use a buffered diluent. Decomposition of the anesthetic can result from improper storage. The pH should be greater than 5. When the pH falls below 5, decomposition to toxic substances can occur. If pH falls, or if crystallization or any discoloration is noted, the anesthetic should be discarded as it can cause death within 24 hours of injection.

Tribromoethanol is given as an intraperitoneal (IP) injection at a dose of 240 - 400 mg/kg body weight. Ketamine and xylazine are given together in the same syringe and are also usually administered IP. Dosage ranges for ketamine and xylazine are 60 - 100 mg/kg and 5 - 10 mg/kg respectively. After anesthetizing the animal, the corneas should be protected with an ophthalmic lubricant. To avoid contamination of the lubricant, do not touch the tip of the tube to the skin or eye surface.

While waiting for the mouse to become anesthetized, the sterile surgical instruments should be prepared. If using steam sterilized instruments, the pack can be opened. If cold sterilization with an agent such as glutaraldehyde is used, the instruments must be rinsed with sterile water, saline, or 70% alcohol as they are removed from the sterilant solution. This step is critical, as cold sterilant is very irritating to tissues. Required exposure times must be followed to ensure sterilization of
instruments. Glass bead sterilizers can also be used to sterilize the tips of the surgical instruments. If using a glass bead sterilizer it is important to remember the following:

- Only the tips of the instruments are sterile.
- The tips of delicate instruments may become damaged during immersion in the glass beads.
- The tips of the instruments become extremely hot and must be allowed to cool before applying them to the skin or other tissues.

Place the sterile instruments on a sterile field. To avoid contamination of the tips of the instruments during the surgical procedure, always keep the tips on the sterile field and pointed in the same direction (Fig. 14).

Figure 14. Placement of surgical instruments.

Once the mouse is anesthetized, the fur is removed from the surgical site by shaving. This should be done in a location different than that used for performing the surgeries. Hair can also be removed from the surgical site by gently plucking the fur.

The surgical site is prepared with alternating scrubs of an iodophor or chlorhexidine and 70% alcohol. Cotton-tipped swabs or 2x2 gauze sponges may be used. It is important to avoid excessively wetting the mouse, as this will lead to hypothermia and anesthetic complications. Once prepped, the mouse is placed on several 4x4 gauze sponges or a small heating pad (e.g., instant heat
device, circulating hot water blanket) to provide warmth and relocated to the surgery station. Sterile surgical gloves are preferred over non-sterile. Some institutions allow use of non-sterile gloves if the gloved hand never touches the prepped surgical field, and only the sterile tips of the instruments are used to handle the animal’s tissues. A sterile surgical drape should be laid over the animal.

**Embryo Implantation**

The mouse is positioned in lateral recumbency. An incision is made through the skin and abdominal musculature. The ovary is exteriorized, a serrafine clip is placed on the ovarian fat pad in preparation for embryo implantation (Fig. 15), and the mouse placed on a pre-warmed microscope surface.

![Figure 15. Drape placed and ovary exteriorized.](image)

The embryos, one-cell, two-cell, or blastocysts, which have been collected and maintained in sterile conditions, are loaded into the pipette. Note in Figure 16 how the tip of the pipette is resting in such a way as to prevent the tip from becoming contaminated. Also notice that there is a break between the pipette and the suction tube to prevent accidental aspiration. A filter is placed in the apparatus to protect the eggs from contamination, particularly if mouth pipetting is utilized. Although mouth pipetting is prohibited at some institutions, it is still commonly practiced for this procedure. One-cell or two-cell embryos are implanted into the infundibulum; blastocysts are implanted into the proximal uterine horn.
Figure 16. Avoiding contamination of the pipette.

Sterile ophthalmic surgical spears (Fig. 17) work well to blot blood or fluid from the surgical site. Sterile gauze sponges or cotton-tipped applicators may also be used. The abdominal musculature is closed with an absorbable suture. Note in Figure 18 how only instruments are used to handle the tissue. After the abdominal wall has been closed, one to two drops of a long acting local anesthetic such as 0.25% bupivacaine or ropivacaine should be applied to the surgical wound as an analgesic. The skin is closed with a non-absorbable suture or surgical staples. Alternatively, cyanoacrylate surgical adhesive may be used as to close the skin. In a high throughput transgenic facility it is preferred that one person prepare the mouse, one person makes the skin incision and closes it, and a third person does the implanting of embryos. This streamlines the process and keeps the risk of contamination to a minimum.
The mouse is placed on a warming device (e.g., slide warmer, circulating hot water blanket) for recovery from anesthesia. If postoperative analgesia is being administered it should be given now. Once the mouse begins to awaken from the anesthesia, it is placed in the pre-warmed recovery cage. It is important to refrain from placing recovering animals directly onto bedding to avoid the risk of choking on bedding material during the recovery phase. Throughout the recovery and post-surgical convalescent period, mice must be closely monitored for the need for additional analgesia. The cage card or a "special observation" card should be marked with the surgery date, so that one knows when to remove the wound closures and when to expect the birth of pups. Wound closures should be removed in 10 - 14 days.

Since multiple surgeries are often performed each day, the instruments should ideally be re-sterilized by one of the methods described earlier. At a minimum, they should be rinsed with 70% alcohol between mice. This will disinfect but not sterilize the instruments. A glass bead sterilizer is ideal for re-sterilizing the tips of the instruments between surgeries. If you have had to handle another mouse to anesthetize and prepare it, you should don a new pair of sterile gloves before performing the next surgery.

These guidelines have been developed based on best practices and commonly used techniques and procedures that have proven successful. By following these guidelines, mice should recover quickly, have minimal if any anesthetic and surgical complications, and successfully deliver healthy pups.

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