Georgia State University
Institutional Animal Care and Use Committee (IACUC)

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It is the responsibility of the Georgia State University (GSU) Institutional Animal Care and Use Committee (IACUC) to ensure judicious and humane use of animals used in its teaching and research programs that is consistent with federal requirements.*

SURGICAL PROCEDURES GUIDELINES

GONADECTOMY: CASTRATION USING SCROTAL METHOD

1. The animal is anesthetized and placed in dorsal recumbency with the tail toward the surgeon.
2. The abdominal and scrotal area is shaved and cleaned with surgical scrub according to the GSU Aseptic Technique for Animal Surgery Guideline.
3. A small median incision, about 1 cm, will be made through the skin at the tip of the scrotum.
4. The testicle is exteriorized by blunt dissection of the tissue, while the testicle is inside the parietal vaginal tunic (commonly referred to as a "closed" castration).
5. A ligature is placed using absorbable suture around the parietal vaginal tunic proximal to the testicle. The parietal vaginal tunic is cut distal to the ligature to remove the testicle.
   *If the parietal vaginal tunic is inadvertently opened during the procedure (commonly referred to as an "open" castration), ligate the vas deferens and blood vessels using cautery or absorbable suture. Cut distal to the suture, to remove the testicle. In the case of the open castration, a final ligature will then be placed around the parietal vaginal tunic so that an otherwise open communication between the abdominal cavity and the scrotum is closed.
6. The scrotal incision is closed with skin glue or wound clips.

GONADECTOMY: CASTRATION USING ABDOMINAL METHOD

1. The animal is anesthetized and placed in dorsal recumbency with the tail toward the surgeon.
2. The abdominal area is shaved and cleaned with surgical scrub according to the GSU Aseptic Technique for Animal Surgery Guideline.
3. A midline skin and muscle incision are made cranial to the penis.
4. One of the testicles, with its associated epididymis, vas deferens, and spermatic blood vessels is exteriorized by gently grasping the peri-testicular fat (associated fat) located in the caudal abdomen.
5. The vas deferens and spermatic blood vessels are cauterized on mice, and cauterized or ligated with absorbable suture on larger rodents (i.e. rats, hamsters). The vas deferens and spermatic blood vessels are transected distal to the site of cautery or ligature.
6. The remaining tissue is replaced into the abdominal cavity.
7. The procedure is repeated contralaterally.
8. The abdominal muscle wall incision is closed with absorbable suture in a simple interrupted pattern.
9. The skin is closed with wound clips.

**INTRACEREBRAL MICROINJECTION**
1. The animal is anesthetized and placed in ventral recumbency.
2. The head area is shaved and cleaned with surgical scrub according to the GSU Aseptic Technique for Animal Surgery Guideline.
3. A small single incision will be made on the midline on top of the head to reveal the underlying bone fissures. The position of the cannulae will be determined via the use of a stereotaxic atlas.
4. A hole through the skull will be trephined to allow insertion of the guide cannulae to the appropriate depth.
5. The cannulae will be anchored to the skull with strategically placed jeweler’s screws, cyanoacrylate ester glue base and then dental acrylic.
6. The incision will be closed with wound clips or non-absorbable suture being careful not to impair the eyelids.
7. Microinjections will be made through an infusion cannula that penetrates past the tip of the guide cannula. Between injections, a sterile obturator will be inserted in the guide cannulae.

**OSMOTIC MINIPUMP IMPLANTATION: SUBCUTANEOUS INFUSIONS**
1. The animal is anesthetized.
2. The area is shaved and cleaned with surgical scrub according to the GSU Aseptic Technique for Animal Surgery Guideline.
3. An incision is made in the skin, and the skin is bluntly dissected away from the muscle layer creating a pocket for the minipump.
4. The sterile minipump is inserted subcutaneously.
5. The incision is closed with wound clips.
   *This may have to be repeated for long duration experiments to replace old pumps with new ones.

**OSMOTIC MINIPUMP IMPLANTATION: INFUSION INTO A BRAIN CANNULA**
1. The animal is anesthetized and placed in ventral recumbency.
2. The head and dorsal area of the neck are shaved and cleaned with surgical scrub according to the GSU Aseptic Technique for Animal Surgery Guideline.
3. An incision is made in the skin over the dorsal neck, and the skin is bluntly dissected away from the muscle layer creating a pocket for the minipump.
4. The sterile minipump is inserted subcutaneously.
5. The pump is connected via sterile tubing tunneled under the skin to a head cannula (head cannula surgical placement described elsewhere).
6. The incision is closed with wound clips.
   *This may have to be repeated for long duration experiments to replace old pumps with new ones.

OSMOTIC MINIPUMP IMPLANTATION: INFUSION INTO A VESSEL
1. The animal is anesthetized.
2. The area is shaved and cleaned with surgical scrub according to the GSU Aseptic Technique for Animal Surgery Guideline.
3. An incision is made in the skin, and the skin is bluntly dissected away from the muscle layer creating a pocket for the minipump.
4. The sterile minipump is inserted subcutaneously.
5. The pump is connected via sterile tubing tunneled under the skin to a cannulated vessel (vessel cannulation surgical placement described elsewhere).
6. The incision is closed with wound clips.
   *This may have to be repeated for long duration experiments to replace old pumps with new ones.

OVARIECTOMY
1. The animal is anesthetized and placed in ventral recumbency.
2. The dorsum is shaved and cleaned with surgical scrub according to the GSU Aseptic Technique for Animal Surgery Guideline.
3. A small incision is made in the skin approximately half way between the shoulder-blades and the base of the tail.
4. The skin is bluntly dissected away from the muscle to allow for movement of the skin incision opening to either side laterally.
   *Alternatively, bilateral skin incisions are made in each flank.
5. The muscle layer of the abdominal wall is gently elevated with forceps through the opening of the skin incision about 2/3 of the way down the side of the body. An incision through the muscle of the abdominal wall is made either with a scalpel blade or with scissors. The ovary, surrounded by fat, should be directly underneath the incision.
6. The ovary is exteriorized through the incision by grasping the peri-ovarian fat (fat around the ovary). Care is taken to avoid touching the ovary itself as this could allow tissue remnants of the ovary to detach from the ovary and potentially establish themselves as viable functioning ovarian tissue that attaches to the abdominal body wall.
7. The ovary is identified within the peri-ovarian fat. The proximal uterine horn is also identified. The proximal uterine horn is cauterized or ligated with absorbable suture being careful to ensure that this occurs below the entirety of the ovary. The proximal uterine horn is transected such that the area of cauterization or ligation remains with the uterine horn which is returned to the abdominal cavity.

8. The muscle wall incision is closed with absorbable suture using a simple interrupted pattern.

9. The skin incision is closed with wound clips.

TRANSCARDIAL PERFUSION (TERMINAL PROCEDURE)

1. The animal will be anesthetized and placed in dorsal recumbency.
2. The hair is wet down with alcohol (or hair will be clipped).
3. An incision in the abdominal cavity is made just below the rib cage and extended dorsally on both sides. Scissors or a sharp scalpel blade is used to cut along the dorsal aspect of the ribs toward the cranial aspect of the thoracic cavity to expose the thoracic cavity.
4. A needle is inserted into the left ventricle and a small incision is made in the right atrium. Note: if a blunted needle is used for this procedure, then one may find it facilitative to make a small nick in the wall of the left ventricle (not all the way through) as this will make it easier to insert the needle particularly in larger rodents such as hamsters and rats. If using a blunted needle in the left ventricle one can also “feed” the needle into the ascending aorta as it exits the left ventricle. In either case, one may find it facilitative to clamp the needle in place prior to beginning perfusion.
5. The perfusion pump is started, typically with saline. Once the liver is blanched, the switch is made from saline to fixative.
6. Perfusion with fixatives will occur within the confines of a fume hood or backdraft station.

* If upper body perfusion is only needed, one may wish to clamp the descending aorta distal to the liver.

SILASTIC CAPSULE IMPLANTATION:

1. The animal is anesthetized and placed in ventral recumbency.
2. The dorsal scapular area is shaved and cleaned with surgical scrub according to the GSU Aseptic Technique for Animal Surgery Guideline.
3. A 0.5 cm incision is made in the skin, and the skin is bluntly dissected away from the muscle layer creating a pocket for the capsule.
4. The sterile silastic capsule is inserted subcutaneously.
5. The incision is closed with wound clips.

VESSEL CANNULATION:

1. The animal is anesthetized and placed in dorsal recumbency.
2. The neck area is shaved and cleaned with surgical scrub according to the GSU Aseptic Technique for Animal Surgery Guideline.
3. An incision is made in the skin on the ventral surface of the neck and blunt dissection through the muscle is utilized. The external jugular vein (at least a cm) is then exposed.
4. A silk suture is tied tightly at the anterior end of the vein. A very loosely tied suture is placed around the posterior end of the vein (do not close the knot).
5. A “catheter introducer” is inserted into the vein between the two sutures.
6. The catheter (typically Silastic tubing) is inserted into the vein and advanced towards the heart.
7. The posterior suture is closed around the vein and catheter to fix the catheter in place. Another suture is placed around the vein and catheter nearer the anterior end of the vein.
8. The other end of the catheter is tunneled subcutaneously to exit the animal through a small skin incision on the dorsal surface of the neck.
9. The catheter (where it exits the incision in the dorsal neck) is encased in dental cement and anchored to a square of durable mesh for subcutaneous placement in the mid-scapular region to stabilize the catheter and keep it from moving. Care is taken to assure that there is enough catheter length to allow for animal movement and growth so as not to dislodge the catheter from the vein.
10. The patency of the catheter is checked by gently aspirating blood from the vessel and then flushed with saline, followed by a heparin/saline (hep-lock).
11. The end of the catheter is capped with a stainless-steel pin.
12. The skin is closed with wound clips.

Pertinent Regulations*
U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training
Public Health Service Policy
Guide for the Care and Use of Laboratory Animals
Animal Welfare Act (AWA) and AWA Regulations