

Description of procedure:

For all gonadectomy procedures:

The animal is weighed and anesthetized following <u>UCSF Rodent Anesthesia Guidelines</u>. Anesthesia and analgesic drugs are administered as per Section I. Agents in the IACUC protocol. A surgical plane of anesthesia and peri-operative analgesia are required. Multimodal analgesia is expected (e.g., local anesthetic, NSAID, and opioid) unless another method is approved in the IACUC protocol. The surgeon will follow <u>IACUC</u> <u>guidelines for aseptic survival surgery</u>.

A. Ovariectomy

- 1. Ovaries are typically approached by two separate flank incisions. Animal may be placed in lateral or ventral recumbency as shown in [Fig 1] or [Fig.2] and prepared for surgery.
- 2. The skin incision is made perpendicular and ventral to the spine, approximately midway between the last rib and iliac crest (hip). Incision should be approximately 5 mm in mouse and approx. 10 mm in a rat.
- 3. Following skin incision, an incision is made through the abdominal wall.
- 4. Hold the abdominal wall tissue with forceps, while a second set of forceps is used to spread the incision so the ovarian fat pad can be identified.
- 5. Grasp and exteriorize the ovarian fat pad.
- 6. The ovary may be excised with ligation, or cautery:
 - a) Ligation: A ligature is placed at the boundary between the oviduct and uterus.
 - b) Cautery: Use cautery pen or heat forceps in bead sterilizer then place on uterine horn at oviduct to simultaneously crush and cauterize.
- 7. Remove ovary, and assure hemostasis has been achieved before allowing the fat pad to return to the abdomen.
- 8. Muscle layer is closed, usually with absorbable suture. Skin is closed with monofilament suture or wound clips.
- 9. Once the first incision is closed, the second side treated as the first.
- 10. Anesthetic recovery follows UCSF Rodent Anesthesia Guidelines.

B. Orchiectomy

Two approaches are possible: an abdominal approach, or a scrotal approach.

For mice the abdominal approach is preferred over the scrotal approach; in mice the inguinal canals remain open for life, so use of the scrotal approach can lead to herniation of the epidydimal fat pad and abdominal contents in this species.

Abdominal approach (preferred approach for mice):

- 1. Animal is placed in dorsal recumbency. A single 5mm skin incision is made on the ventral midline at the caudal abdomen, just cranial to the preputial glands and extending toward the umbilicus.
- 2. A second incision is made through the linea alba. Using gentle pressure on the abdomen, press both testes down from the scrotum into the abdomen to access them through the incised area.
- 3. The testicular fat pad for one side is exteriorized using blunt forceps on to a 2x2 gauze placed adjacent to the incision.
- 4. The testicle is removed by placing a hemostat caudal to the testes and epididymis across the testicular cord (contains blood vessels and vas deferens). A ligature (absorbable suture is preferred) is placed proximal to the hemostat. The surgeon then removes the testes and epididymis, releases the hemostat, and makes sure no bleeding occurs. Repeat for the second testicle.
- 5. Once hemostasis is assured the epididymal fat pad is replaced into the abdomen and the incision is closed in two layers with suture.

Scrotal approach (rats):

- 1. A single midline incision is made over the distal aspect of the scrotum [Fig 3].
- 2. Both testes can be reached through the same incision. Make a 5 mm incision through the scrotal skin and the underlying tunica
- 3. Using gentle pressure on the abdomen, press both testes down into the scrotum.
- 4. The testicular fat pad on the one side is exteriorized using blunt forceps.
- 5. The testicle is removed, and a hemostat is placed caudal to the testes and epididymis across the testicular cord (contains blood vessels and vas deferens). A ligature (absorbable suture is preferred) is placed proximal to the hemostat. The surgeon then removes the testes and epididymis, releases the hemostat, and makes sure no bleeding occurs. Repeat for the second testicle.
- 6. Once hemostasis is assured the epididymal fat pad is replaced into the abdomen. The muscular layer should be closed with a single incision, prior to closing the skin with suture.

Agents: This procedure requires appropriate anesthetic and analgesic agents. All agents administered to animals should be listed in the "Agents" section I of the RIO application.

Adverse Effects: Adverse effects of hemorrhage, herniation, pain and infection should be listed in Section J of the RIO application.

Figure 1. Flank incision bilateral approach to the ovaries, animal in lateral recumbency



Figure 2. Flank incision bilateral approach to the ovaries, animal in ventral recumbency



From Souza, V.R. et.al (2019). Description of Ovariectomy Protocol in Mice. In: Guest, P. (eds) *Pre-Clinical Models. Methods in Molecular Biology*, vol 1916. Humana Press, New York, NY. https://doi.org/10.1007/978-1-4939-8994-2_29

Figure 3A. Incision into the rat scrotum

Figure 3B. Removal of the rat testisi after ligation of the spermatic blood vessels and vas deferens



From H.B. Waynforth, Experimental and Surgical Technique in the Rat, Academic Press, 1980.