



STANDARD OPERATING PROCEDURE 307 RODENT MAMMARY FAT PAD INJECTION

1. PURPOSE

This Standard Operating Procedure (SOP) intends to describe the procedure for mammary fat pad injection surgery in rodents.

2. RESPONSIBILITY

Principal investigator (PI) and their research staff.

3. MATERIALS

- 3.1. Analgesics
 - 3.2. Anesthetic
 - 3.3. Sterile ophthalmic ointment
 - 3.4. Electric clipper or depilatory cream
 - 3.5. Sterile gauze
 - 3.6. Antiseptic solution for skin (e.g., chlorhexidine 4% solution or povidone-iodine solution used alternatively with 70% alcohol or 0.5% chlorhexidine in 70% alcohol solution)
 - 3.7. Heating pack or warming pad.
 - 3.8. Sterile isotonic saline solution
 - 3.9. Sterile cotton-tipped swabs
 - 3.10. Sterile surgical instruments
 - 3.11. Hot bead sterilizer
 - 3.12. Surgical tissue glue, absorbable sutures (e.g., 5-0 Assucryl), or wound clips
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4. PROCEDURE

- 4.1. Refer to Rodent Surgery SOP.
- 4.2. Document the details of the surgical procedure in the Rodent Procedure Log or with the BGU Preclinical Management System.
- 4.3. Administer analgesia to recipient animals as per Rodent Analgesia SOP.
- 4.4. Anesthetize the donor (if applicable) and recipient animal as per Anesthesia SOP.

- 4.5. Apply ophthalmic ointment in both eyes of the recipient animal to prevent corneal desiccation. Reapply as needed.
- 4.6. If applicable, obtain donor animal tissues (small tumor pieces) for implantation. Place the sample in sterile PBS until implantation—Euthanize the donor with CO₂.

Note: the donor animal is anesthetized instead of euthanized before tumor collection to maintain the sterility of the samples and minimize the time to transplant.
- 4.7. Remove hair over the area surrounding the animal's abdominal (#4) mammary gland using a clipper or depilatory cream. Remove loose hair with gauze.
- 4.8. Wipe the skin surface with 70% alcohol followed by 4% chlorhexidine or povidone-iodine solution.
- 4.9. Mammary Fat Pad Injection:
 - 4.9.1 Place the recipient animal in dorsal recumbency.
 - 4.9.2 Make a 0.5cm incision between the abdominal (#4) mammary gland/nipple and the midline. Alternatively, the incision can be made in the flank, cranial to the abdominal (#4) mammary gland/nipple.
 - 4.9.3 Gently separate the skin from the underlying fascia using a sterile cotton swab or blunt dissection. Care should be taken not to damage the peritoneal cavity.
 - 4.9.4 Injection of cell suspension:
 - 4.9.4.1 Locate the mammary fat pad, grasp it at its base, and stabilize it using jewelers or micro-dissecting forceps.
 - 4.9.4.2 Inject cells directly into the fat pad in a maximum volume of 100µL
 - 4.9.5 Implantation of tissue fragments:
 - 4.9.5.1 Locate the mammary fat pad, grasp it at its base, and stabilize it using jewelers or micro-dissecting forceps.
 - 4.9.5.2 Prepare a pocket in the fat pad by carefully inserting the closed points of a jeweler's forceps into the fat pad.
 - 4.9.5.3 Remove forceps points from the fat pad and transplant donor tissue into the prepared pocket. Tissue fragments should exceed 3x3x3mm.
 - 4.9.6 Apply local topical analgesic to the incision.
 - 4.9.7 Hold the edges of the incision together with forceps and use one drop of tissue glue to close the skin. Alternatively, suture the incision or use wound clips.
 - 4.9.8 Repeat on the contralateral side.
- 4.10 Disinfect the instruments between each animal by dipping them in a hot glass bead sterilizer for approximately 30 seconds after removing any blood and debris (let cool completely).
- 4.11 Allow animals to recover in a clean cage. Provide supplemental heat for approximately 30 minutes and monitor the animals until they fully recover before returning them to their housing room.
- 4.12 Administer analgesics post-operatively as per Rodent Analgesia SOP.

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Approved by the BGU Animal Policy and Welfare Oversight Committee