Hind Limb Ischemia

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Summary:

This is a mouse model of hind limb ischemia, a technique involving an interruption in the arterial blood supply to tissue in the hind limb. This model is used to study peripheral artery disease and vascular remodeling.
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Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Ketamine	Patterson veterinary	07-803-6637
Xylazine	Patterson veterinary	07-869-6707
Isoflurane 1-3%	Patterson veterinary	07-893-1389
Buprenorphine	Patterson veterinary	07-891-9756
Meloxicam	Patterson veterinary	07-893-1368
6-0 Proline suture	Patterson veterinary	07-824-3204
7-0 Silk suture	Patterson veterinary	07-824-1501

Protocol:

WARNING HAZARDOUS CONDITION WARNED AGAINST. This comment describes a hazardous condition to which the technician may be exposed in the performance of this protocol. It also contains directions on how to avoid or minimize the danger. Warnings are always and only used for personnel safety, and precedes the first step that will expose

Expected procedure duration:

30 minutes

Adequacy or depth of anesthesia is monitored by:

Respiratory Rate and Toe Pinch

Frequency of anesthesia depth assessment:

At the start of surgical procedure, a toe or ear pinch can be used to assess the depth of anesthesia. Visual monitoring should be performed thought-out the procedures, as well as toe/ear pinches.

Deviations from expected behavior Should be noted.

Anesthesia Regimen:

Ketamine (80-100mg/kg), Xylazine (5-20mg/kg) or Isoflurane 1-3%

Pre-surgical Analgesics:

Approximately 30 minutes prior to undergoing the surgical procedure, mice receive an S.C. injection of Buprenorphine (0.05mg/kg) and Meloxicam (5mg/kg).

Surgical prep:

Aseptic technique will be maintained by:
Clipping/shaving fur around incision site, Sterile Instruments.

Isoflurane, heating pad, forceps, scissors, needle driver, eye ointment, 6-0 Prolene, 7-0 silk suture, 1 ml-syringes, 0.9% NaCl, Sterile gloves, Povidone-idoine, 70% ETOH

Surgical Procedure:

1. Anesthetize the mice and ensure depth of anesthesia with a toe pinch.
2. Place the mice supine on the heating pad.
3. Remove the hair from 0.5 cm above the elbow to 0.5 cm below the knee joint of surfaces to be joined.
4. Prep the surgical field with 70% isopropanol as well as Betadine solution and drape the mice.
5. Apply ointment to animal's eyes.
6. Confirm depth of anesthesia with a toe pinch.
7. Make an incision in the skin from the medial thigh towards the knee.
8. Blunt dissect away subcutaneous fat tissue to reveal the underlying femoral artery.
9. Pierce the membranous femoral sheath to expose the neurovascular bundle. Dissect and separate the femoral artery from the femoral vein and nerve from the inguinal ligament to the epigastrica.
10. Place 2 7-0 silk sutures around femoral artery 2mm apart and ligate. Divide the femoral artery between the 2 ligating sutures.
11. Close the skin incision using 6-0 Prolene suture.
11. Injection 0.5 ml of 0.9% NaCl subcutaneously to each mouse to prevent dehydration.

Surgical Procedure (alternate): Severe ischemic model

2. Anesthetize the mice and ensure depth of anesthesia with a toe pinch.
2. Place the mice supine on the heating pad.
3. Remove the hair from 0.5 cm above the elbow to 0.5 cm below the knee joint of surfaces to be joined.
4. Prep the surgical field with 70% isopropanol as well as Betadine solution and drape the mice.
5. Apply ointment to animal's eyes.
6. Confirm depth of anesthesia with a toe pinch.
7. Make an incision in the skin from the medial thigh towards the knee.
8. Blunt dissect away subcutaneous fat tissue to reveal the underlying femoral artery.
9. Pierce the membranous femoral sheath to expose the neurovascular bundle. Dissect and separate the femoral artery, femoral vein and nerve from the inguinal ligament to the sapheno-popliteal bifurcation.
10. Ligate the femoral artery using 7-0 silk sutures between the superficial epigastric artery and the sapheno-popliteal bifurcation.
11. Close the skin incision using 6-0 Prolene suture.
11. Injection 0.5 ml of 0.9% NaCl subcutaneously to each mouse to prevent dehydration.

Post-procedure Analgesics:

Buprenorphine (0.05mg/kg) every 12 hours, for 72 hours post-op.
Meloxicam (5mg/kg) every 24 hours, for 72 hours post-op

Post-procedure Monitoring:

Mice are monitored 2x daily for the first 5 days post the surgery. Thereafter, mice are monitored at least 3x per week.