

Scisense PV Surgical Protocol

Mouse Femoral Artery Acute Blood Pressure Measurement

APPLICATION BASICS

Site:	Femoral artery
Species:	Mouse
Body Weight:	20- 50 grams
Duration:	Acute

CATHETER

Size:	1.2F
Type:	Pressure
Catalog #:	FTH-1211B-0018

SYSTEM	SP200, SP430
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Application

Invasive femoral artery blood pressure measurement can be used to determine peripheral mean arterial pressure (MAP) and assess blood supply to the leg. Performing intravascular pressure measurement allows precise local measurement of peak, systolic, and diastolic pressure; along with more detailed analysis of systolic or diastolic durations, developed pressure, isovolumetric times, and pulse height.

Anatomical Landmarks

The femoral artery in mice is located in the area of medial thigh in direct proximity of muscle groups of mm. pectineus and adductor. The surgical dissection is done in dorsal recumbency.

Pre-Surgical Preparations and General Anesthesia

See Research Equipment Sources (RL-90-tn) for recommended equipment suppliers. Prepare an area for scrubbing in a separate location from where the surgical operation will take place. It is best to find a low-traffic area. Clean surfaces using disinfectants with low reaction to organic materials (e.g. Phenolics -- Lysol, TBQ).

Basic surgical supplies include a sterile surgical instrument pack and sterile supplies (i.e. drapes, 4"x4" gauze squares, Q-tips, disposable high-temp fine tip cautery, 5 ml syringes, saline rinse, tray, gloves, mask, head bonnet and sterile suture packs). In addition, a glass bead sterilizer, heating water blanket or approved electrical heating/feed-back control unit should be used. Heat lamps are not ideal for body temperature maintenance and can often be a source of electrical noise/interference. Delicate rodent surgical instruments should be inspected for damage before sterilizing.

Set up the surgical microscope (interpupillary distance, check light bulbs, adjust to check magnifications), organize the surgical table and fine-tune the surgical stool to a comfortable setting where the triangular position can be reached (both feet touching the ground with both arms comfortably resting on the surgical table). Turn on the glass bead sterilizer.

Prepare 0.9% saline or a similar isotonic fluid and pre-warm the solution if it will be given pre-operatively. When a decision is made to use pre-warmed sterile isotonic fluids subcutaneously it is also suggested to use a preventive analgesia.

Before inducing anesthesia make sure to record weight, age, sex, strain, colony history and health status of each mouse, and determine whether animals have had enough acclimatization time (usually 3 days post arrival). Check mouse respiratory rate (80-240 breaths/min), heart rate (500-600 beats/min) and temperature (37.1-37.5°C).

Mouse Acute Femoral Pressure Measurement Cont.

Pre-Surgical Preparations and General Anesthesia Cont.

Shave the area of medial thigh while on warming pad, using small animal clippers (e.g. ChroMini cordless clipper). Remove remaining hair from the surgical area using a depilatory cream (e.g. Nair). Apply surgical scrub alternating between disinfectant (e.g. iodophores, chlorhexidines) and alcohol. Please remember: Iodophores will inactivate a wide range of microbes, however literature describes their reduced activity in the presence of organic matter.

Use gauze squares for scrubbing. Scrubbing should begin along the incision line and extend outward and never from outward (dirty) towards the center (clean). Always scrub a larger surface area than surgical field. Do not wet large area of skin or fur with alcohol to avoid hypothermia. Consider using drapes to maintain a sterile field and preserve body temperature.

Pre-anaesthetize mouse for the femoral artery dissection with 3-4% Isoflurane (Forane) mixed with driving gas (Oxygen) 0.5 L/min inhaled in Plexiglas induction chamber with lid. It is important not to disturb mouse during induction. Apply an ophthalmic ointment to both eyes following induction of anesthesia to prevent corneal drying.

When connected to the face mask, inspect breathing pattern, color of membranes and capillary refill time. If feasible, use pulse oximetry. We have found that Isoflurane produces an excellent long-term controllable anesthesia. Adequate anesthesia is accompanied by loss of muscle tone and by loss of reflexes (e.g. corneal, pinnae and pedal).

Regulate post-induction anesthesia with animal placed on a warming pad (38°C) in a supine position, with the upper and lower extremities attached to the table with surgical tape, then maintain mouse on 2% Isoflurane by using face mask.

Prior to surgery, soak the tip of the Pressure Catheter FTH 1211B-0018 in 0.9% isotonic saline for ~ 20 minutes before insertion into the femoral artery. Connect the SP200 or SP430 system to the data acquisition software, ensuring that Pressure channel is calibrated. After soaking, adjust the pressure balance to zero for atmospheric pressure (Please see Pressure Sensors Calibration technical note SP-1-tn).

Other methods of anesthesia may be used. Be sure to consider cardiovascular impact of anesthetic choice. Please adhere to your institutions guidelines for anesthesia and pain management. See Rodent Anesthesia Guidelines (RL-67-tn) for more considerations.

Surgical Approach

FEMORAL ARTERY DISSECTION

Secure animal in supine position on the heating pad. Using sharp scissors, starting immediately in the medial area of thigh make a straight incision about 2 cm long, from the knee towards the medial thigh. Make the incision straight and while lifting the skin with thumb forceps, keep the scissor tips up. Using blunt Metzenbaum scissors or medium hemostats bluntly dissect an underlying subcutaneous tissue from skin (Fig.1). Instruments are parallel to the tissues bluntly dissecting with tips closed then wide open, gently separating skin from underlying tissue circumferentially around the entire incision wound.

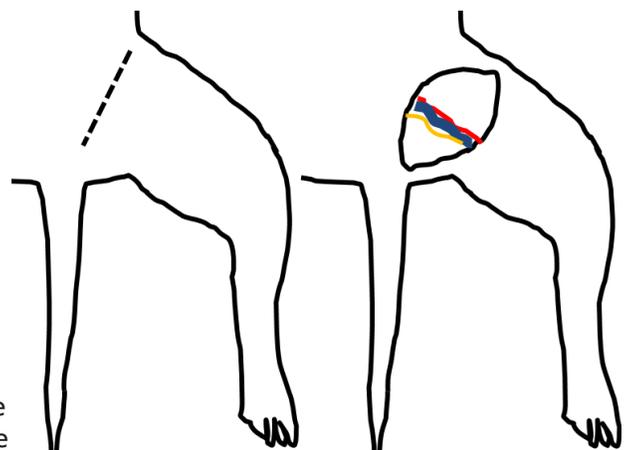


Fig. 1: Dissection of the femoral artery. A) position of the initial skin cut and B) schematic location of the fem. artery (red), fem. vein (blue) and fem. nerve (yellow). Larger vein covers the artery during the initial blunt probing and dissection.

Mouse Acute Femoral Pressure Measurement Cont.

Minor bleeding can be stopped by Q-tips or by pre-made spear shaped nitrocellulose sponges (Harvard app, QC). Keep the area moist with warm sterile saline or PBS. Gently separate via blunt dissection to expose underlying muscular layer and use retraction for visualization of the underlying femoral nerve, vein and artery. At this stage you can use fine jewelers forceps to separate medial thigh muscles and to also pierce through the membranous femoral sheath (Fig.2).

Dissect free and separate the femoral artery from the femoral vein and nerve in the area close to the inguinal ligament and follow dissection along the vessels using two fine jewelers forceps or you can use a dissecting blunt-tipped spring (e.g. McPherson-Vannas scissors) under direct microscopy visualization via at least 25x magnified field. Dissection along the femoral artery has to be done with caution as the major muscular branch called Murphy's branch has to be avoided as profuse bleeding occurs when forcefully separated. Another branch is located at about halfway down the dissection length on the femoral vein. Ensure that the section of the artery is completely separated from all adjacent tissues to limit unexpected bleeding during the retraction and/or clamping procedures.

Post-successful dissection, pass 10-0 silk suture underneath the femoral artery and ligate the distal end of the femoral artery (towards the knee) using double knots (Fig.3) and create tension on the distally placed suture end. Keep in mind a longer isolated segment will significantly improve chances for successful Catheter introduction. Avoid excessive pressure on the vasculature and try to maintain normal vessel geometry. Flush the area using 0.9% isotonic saline or PBS using 27-gauge Angiocath or similar (Fig.4).

Place another larger size silk 7 or 8-0 underneath the segment this time more distal order to leave enough length for the second tie securing the Pressure Catheter in place (Fig.5). Do not use 5-0 or larger size silk as on ligation you are able to twist the long axis of the femoral artery making it more difficult to cannulate. At the same time place microvascular clamps underneath the artery for later help with Pressure Catheter positioning. Then prepare a knot on the 7-0 silk suture (Fig.6).

Use another microvascular clamps to temporary occlude blood flow into the segment and use 30 gauge needle to perforate the vessel using the other underlying clamp to support the needle tip and the Pressure Catheter on insertion (Fig.7-9).

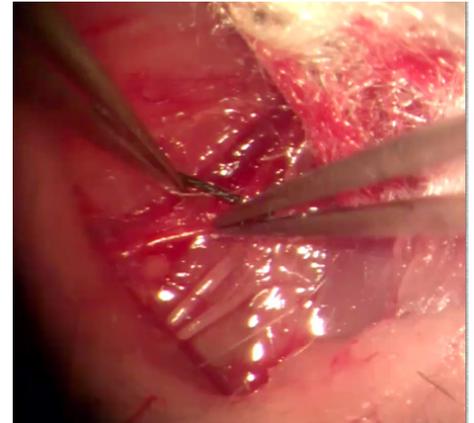


Fig. 2: Separate the thigh muscles and retract the larger femoral vein to locate the artery.

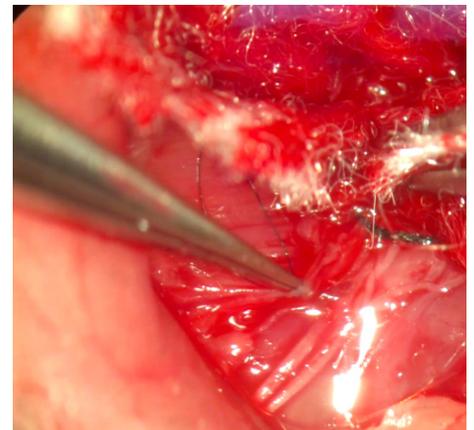


Fig. 3: Place first suture underneath the femoral artery



Fig. 4: Saline flush

Mouse Acute Femoral Pressure Measurement Cont.

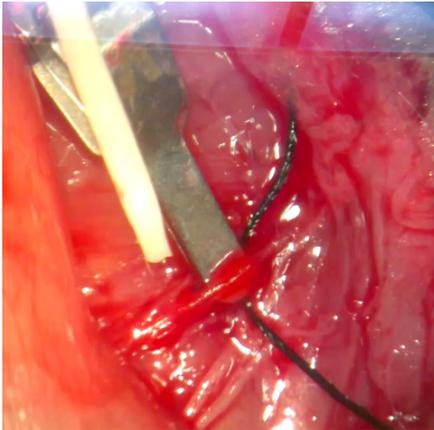


Fig. 5: Place 7-0 silk under the femoral artery and apply suction

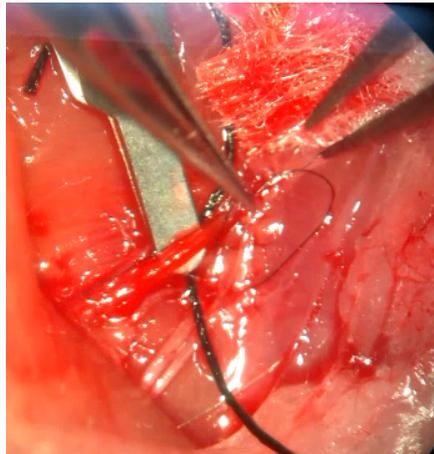


Fig. 6: Tie knots in sutures



Fig. 7: Use a 30g needle to perforate vessel

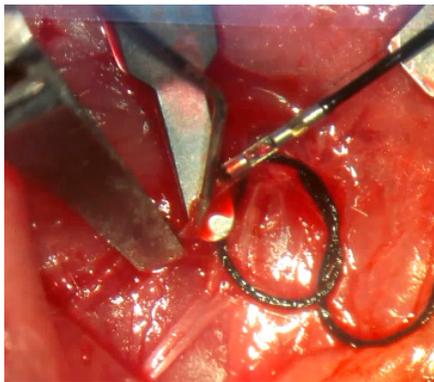


Fig. 8: Position the Pressure Catheter right behind the perforating needle, aligning it with long axis of the arterial segment. Perforating needle is positioned towards the inguinal ligament.

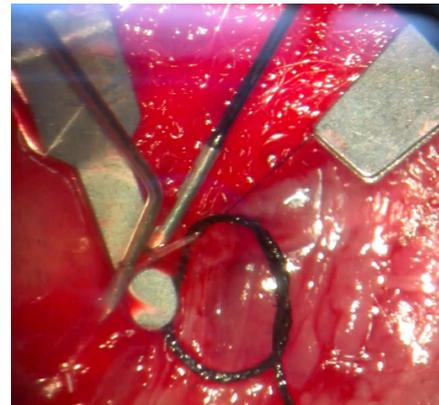


Fig. 9: Fully introduce the needle while at the same time get ready to slide in the Pressure Catheter immediately upon the needle withdrawal. Please note the tension created with 10-0 suture is necessary to help to straighten up the segment before Catheter entry.

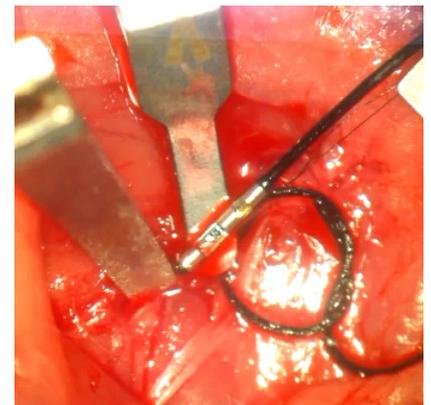


Fig. 10: Insert the Catheter into the femoral artery. On Catheter insertion; Catheter is positioned as close as possible to the microvascular clamp.

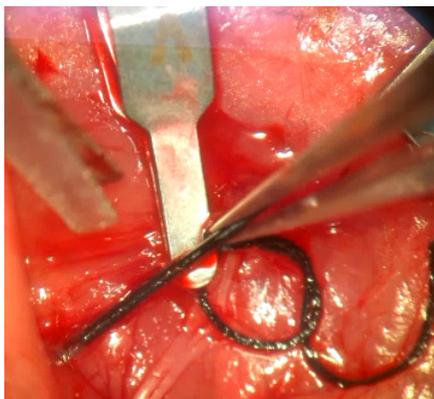


Fig. 11: Fully open the microvascular clamp and as you are removing the clamp push the Catheter fully into the lumen.

Mouse Acute Femoral Pressure Measurement Cont.

CATHETER INTRODUCTION (INSERTION)

Following a successful femoral arteriotomy insert the Pressure Catheter as close to the microvascular clamp as possible (Fig.10). Then use the clamp applicator to lift the clamp off of the segment (Fig.11) and at the same time insert with your non dominant hand the 1.2F Pressure Catheter into the opening, passing the pressure sensor into the area of inguinal ligament. Especially for a novice surgeon, who might take more time to successfully introduce the Catheter, using the microvascular clamp might allow more time for location of the insertion into the collapsed artery, limiting blood loss from catheterization. You might position and tie off the first suture around the Catheter after passing the pressure sensor in order to prevent its slipping out. At the same time, please make sure there is not an excessive resistance present on introduction (vasoconstriction, vessel lumen distortion), which might cause excess bleeding out of the arteriotomy incision on repositioning(s).

Be careful not to damage the Catheter with the forceps tips and hold the Catheter at the same plane as the blood vessel during whole introduction (please see technical note How to optimize Scisense Pressure and PV Catheter Life Span). Ideally there is a very low amount of bleeding post insertion.

CATHETER POSITION ADJUSTMENT

Allow the Catheter to stabilize in the artery for 5-10 min before marking the data file to your start protocol. Catheter positional adjustment needs to be made based on acquired pressure signal. Reposition the Catheter until an optimal position is found to obtain a sinusoid pressure wave (Fig.12). The software is later used to detect and mark ES (end systole), ED (end diastole), N (notch pressure), filling end or max and min DP (Fig.12).

At the end of the experiment, carefully remove the Pressure Catheter by gently pulling it back through the stab wound. Immediately, insert the Catheter tip into 5 ml saline pre-filled syringe. Clean the Catheter as soon as possible according to proper care guidelines to considerably prolong the Catheter's life (CSD 2.06 Catheter Cleaning & Disinfecting Guide).

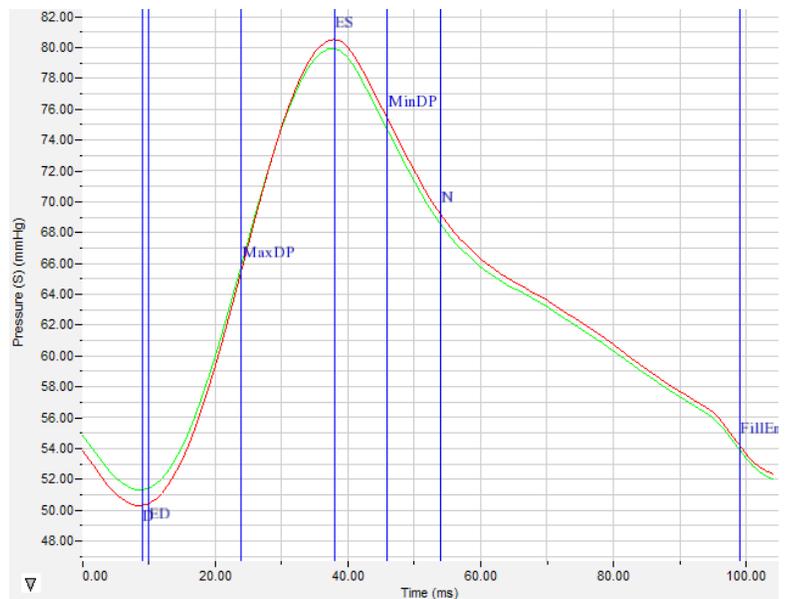


Fig. 12: Mouse Femoral Artery Pressure. Software can determine the points described on the pressure wave: ED (end diastole), ES (end systole), max and min DP (max and min pressure derivatives), N (notch pressure point), FillE (end of filling).



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