Journal of Visualized Experiments

Brachial Artery Catheterization in Swine

--Manuscript Draft--

Article Type:	Methods Article - Author Produced Video
Manuscript Number:	JoVE59365R1
Full Title:	Brachial Artery Catheterization in Swine
Keywords:	brachial artery; catheterization; surgery; arterial pressures, arterial blood gases; swine
Corresponding Author:	Richard R.E. Uwiera, DVM PhD University of Alberta Edmonton, Alberta CANADA
Corresponding Author's Institution:	University of Alberta
Corresponding Author E-Mail:	richard.uwiera@ualberta.ca
Order of Authors:	Richard R.E. Uwiera, DVM PhD
	Toossi Amirali
	Dirk G Everaert
	Trina C Uwiera
	Vivian K Mushahwar,
Additional Information:	
Question	**Response**
Please indicate whether this article will be Standard Access or Open Access.	Standard Access (US$1200)

UNIVERSITY OF ALBERTA

Department of Agricultural, Food and Nutritional Science
Faculty of Agricultural, Life and Environmental Sciences

410 Agriculture/Forestry Centre
Edmonton, Alberta, Canada T6G 2P5
www.afns.ualberta.ca
afns-chair@ualberta.ca
Tel: 780.492.3239
Fax: 780.492.4265

Nov 4, 2018

Dear JOVE,

Please consider the manuscript 'Brachial Artery Catheterization in Swine' for publication. We believe that our video-manuscript provides a detailed, well- produced description of the catheterization of the brachial artery in pigs. This technique is an effective method to measure arterial blood pressures and collect arterial blood for gas measurements in swine. The data indicates that this procedure can be completed relatively quickly with minimal tissue dissection and is a good method to be employed during experimental procedures that involve caudal-ventral, caudal-distal and hindlimbs of experimental animals. Importantly, we believe the JOVE multi-media format is the ideal media forum to present this surgical procedure, a procedure that if not performed correctly can lead to failure.

Please note: Ensuring that the standards of the manuscript and video have met peer review, publishing the article by (or for) the Jan 7-14, 2019 is highly desirable. We would use the methods of this publication for subsequent manuscripts (in preparation) for surgeries in swine involving the catheterization technique.

Author contributions:

- R.R.E. Uwiera (DVM, PhD)- Lead author, co-investigator, veterinary (primary), surgeon, data collection, created and edited the video and drafted the manuscript, intellectual input for scientific advancement, surgery and anatomy expertise
- A. Toossi (BEng, PhD) - data collection and analysis, the manuscript preparation and eting, intellectual input for scientific advancement
- D.G. Everaert (PT, PhD)- Secondary surgeon (surgical assist), data collection, and analysis, intellectual input for scientific advancement
- T.C. Uwiera (MD, Med) –Creating and editing the video and manuscript, intellectual input for scientific advancement, surgery and anatomy expertise for the surgical procedure
- V.K. Mushahwar: (BEng, PhD)- Co-investigator, data analysis, editing the video, drafting and editing the manuscripts, intellectual input for scientific advancement.

TITLE:
Brachial Artery Catheterization in Swine

AUTHORS:
Richard R.E. Uwiera[1], Amirali Toossi[2,3], Dirk G. Everaert[3,4], Trina C. Uwiera[5], Vivian K. Mushahwar[2,3,6]

[1]Department of Agricultural Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada
[2]Neuroscience and Mental Health Institute, University of Alberta, Edmonton, AB, Canada
[3]Sensory Motor Adaptive Rehabilitation Technology (SMART) Network, University of Alberta, Edmonton, AB, Canada
[4]Division of Physical Medicine and Rehabilitation, Department of Medicine, University of Alberta, Edmonton, AB, Canada
[5]Division of Pediatric Surgery, Department of Surgery, University of Alberta, Edmonton, AB, Canada
[6]Division of Physical Medicine and Rehabilitation, Department of Medicine, University of Alberta, Edmonton, AB, Canada

Corresponding Author:
Richard R.E. Uwiera (richard.uwiera@ualberta.ca)
Tel.: 780.492.0107

Email Addresses of Co-Authors:
Amirali Toossi (toossi@ualberta.ca)
Dirk G. Everaert (everaert@ualberta.ca)
Trina C. Uwiera (tuwiera@ualberta.ca)
Vivian K. Mushahwar (vivian.mushahwar@ualberta.ca)

KEYWORDS:
brachial artery; catheterization; surgery; arterial pressures, arterial blood gases; swine

SUMMARY:
The video describes in detail the catheterization of the distal brachial artery in swine. This procedure accurately measures arterial blood pressure and is a simple and fast method to collect samples for arterial blood gas measurements.

ABSTRACT:
The video describes in detail the catheterization of the distal brachial artery in swine. This technique enables researchers to measure arterial blood pressure continuously and collect arterial blood samples to assess arterial blood gas measurements. Arterial blood pressures and arterial blood gases are important physiological parameters to monitor during experimental procedures. In swine, four common methods of arterial catherization have been described, including catherization of the carotid, femoral, auricular, and medial saphenous arteries. Each of

these techniques have advantages, such as ease of access for the auricular artery, and disadvantages that include deep tissue dissection for carotid artery catherization. The described alternative method of arterial catheterization in swine, the catheterization of the distal aspect of the brachial artery, is a rapid procedure that requires relatively minimal tissue dissection and provides information that is in line with data collected from other arterial catheterization sites. The procedure uses a medial approach along an oblique plane of the lower brachium, positioned between the olecranon and the flexor aspect of the elbow joint, and this approach allows researchers the major advantage of unimpeded freedom for procedures that involve the caudoventral, caudodorsal back, or hind limbs of the pig. Due to the location of the upper forelimb of the catheterized vessel and potential challenges of effective homeostasis following catheter removal from the artery, this technique may be limited to non-recovery procedures.

INTRODUCTION:

Surgical intervention is used in experimental research to develop animal models that enhance scientific development. The scientific literature is filled with examples of novel surgical animal models[1-3]. Surgical procedures are a complex process involving not only the manipulation of anatomical structures but also complicated physiological interactions with various drugs required for anesthesia and analgesia. This interplay can induce major changes in physiological processes within the animal and as such requires vigilant monitoring of the animal[4]. Clinically successful surgical outcomes have been associated with measurements of arterial blood gases and arterial blood pressures[5]. These clinical parameters require the ability to measure arterial blood pressure and collect arterial blood effectively, which in turn requires the successful catheterization of an artery[6,7].

Arterial catheterization to collect arterial blood and measure pressure has been used in various animal species[5-21] and in animals at different ages of development[19-21] and has been directed at both recovery (clinical and diagnostic) procedures[4-8] and non-recovery (experimental) procedures[14-18]. Moreover, the ease of arterial access and the location of the artery in the context of the surgical procedure are also important considerations when choosing an artery for arterial blood measurements. For example, the median caudal artery in dogs and the facial artery in horses, as well as the pedal artery in both dogs and horses, are used for diagnostic measurement and monitoring during recovery procedures[6-8]. In contrast, the carotid and femoral arteries are often catheterized in swine for either non-recovery or long-term catheter implantation experiments[14,15,18].

In swine, arterial catherization to measure either arterial blood pressure or collect arterial blood has routinely employed either the carotid, femoral, medial saphenous, or auricular arteries[22,23]. For specialized non-routine procedures, other more unusual arteries have been used, including the subclavian and iliac arteries, to measure the brachial artery anatomical tortuosity[17] and image the aorta[16], respectively. Regardless of which artery is chosen for catherization, each artery has inherent advantages and disadvantages for its use. For instance, the auricular artery is anatomically easy to access, but its use may be limited to its close proximity to the marginal ear veins[11,12]**.** In comparison, the carotid artery is relatively large and robust[24], but it lies deep within the jugular furrow and requires substantive tissue dissection[25]. As such, identifying another artery that could be catheterized to measure arterial pressure and collect arterial blood is warranted. This video and manuscript describe in detail the catheterization of the distal brachial artery in swine, a technique that could be applied to non-recovery procedures. Notably, the pig brachial artery catherization was used to measure arterial blood pressures and arterial blood gas parameters during a lumbar spine surgery with hind limb measurements (the data from this part of the surgery is not presented).

PROTOCOL:

All procedures on experimental animals described in both the video and manuscript were approved by the Institutional Animal Care and Use Committee of the University of Alberta

1. Surgical anesthesia and surgical preparation of the pigs.

1.1. Premedicate 50 kg Landrace-Yorkshire commercial pigs intramuscularly with the anesthetic drug cocktail containing ketamine hydrochloride (22 mg/kg,), xylazine hydrochloride (2.2 mg/kg) and glycopyrrolate hydrochloride (10 µg/kg).

1.2. Set up all equipment involved with monitoring clinical parameters at the end of the table near the pig's head. Ensure the equipment will not restrict access to the pig. Produce accurate arterial blood pressure measurement by placing the pressure transducer in a horizontal plane, level with the heart.

1.3. Anesthetize the pigs with inhaled isoflurane gas (4%–5% isoflurane at 500–1000 mL/min O_2) using a properly sized face mask. Visualize the vocal cords with a veterinary laryngoscope (17–25 cm long straight blade) and apply topical 10% lidocaine spay to the vocal cords for 30–60 s to limit the risk of laryngospasm and airway obstruction.

1.4. Intubate the pigs by inserting a cuffed endotracheal tube (9.0 mm internal diameter (ID)) through the vocal cords and maintain anesthesia with isoflurane gas (0.5%–3.0% isoflurane at 1000–2000 mL/min O_2). Ventilate the pig on a mechanical ventilator (18–22 breaths/min) and ensure all expired anesthetic gas is scavenged and vented outside the surgical suite. Assess the level of anesthesia by jaw tone, and both pedal and palpebral reflex responses.

NOTE: Administer intravenous Lactated Ringer's Solution (LRS, 10–50 mL/kg/h; see step 1.6) to enhance hemodynamic function and reduce isoflurane gas anesthesia induced depression of cardiovascular output in swine[26].

1.5. Secure a pulse oximeter to the mucosal surface of the tongue with medical tape to monitor heart rate and the saturation of peripheral blood oxygenation (SpO_2). Insert a temperature probe approximately 2–4 cm into the nasal cavity to monitor body temperature. Place the pigs on a heated table to maintain normal body temperature (38–40 °C) during the surgical procedure.

1.6. Ensure surgical sterility with proper tissue preparation.

1.6.1. Clean the external surface of the ear to prepare for venous catheterization with 10% povidone-iodine surgical scrub solution and allow the solution to air dry.

1.6.2. With a 20 G, 1 inch intravenous catheter, catheterize a marginal ear vein to deliver either intravenous fluids (LRS; 10–50 mL/kg/h) or the addition of other anesthetic agents.

1.6.3. Enhance pig anesthesia and analgesia if needed for invasive procedures with continuous intravenous remifentanil hydrochloride infusion (0.05–0.14 µg/kg/min).

1.7. Place the pig in a lateral recumbent position and gently extend the front leg approximately 10–12 cm away from the shoulder. Clip the hair on the skin surface of the medial aspect of the brachium (upper forelimb). Landmark the distal brachial artery pulse by palpation.

NOTE: The landmarked location of the artery lies along an oblique plane with the brachium approximately 9 cm from the olecranon, and 5 cm from the flexor aspect of the elbow joint. The brachial artery travels proximally towards the caudal third of the scapula traversing the humerus.

1.8. Similarly to step 1.6, ensure surgical sterility with proper tissue preparation. Clean the skin surface with 10% povidone-iodine surgical scrub solution and allow the solution to air dry. Drape the brachial artery catherization site with four small disposable surgical drapes.

2. Tissue dissection and catheterization of the brachial artery

2.1. Make a 6 cm skin incision with a scalpel blade to expose the underlying tissue. Bluntly dissect with Metzenbaum scissors along the medial surfaces of the biceps brachii, deepening the dissection, until the pulsating artery is identified.

2.2. Use cotton swabs to gently tease away the adventitia from the brachial artery, median nerve and brachial vein; structures that are in close proximity and within the same fascial plane. Gentle dissection is required, importantly ensuring minimal injury to the median nerve during the procedure. The brachial artery lies approximately 2.0–2.5 cm underneath the skin and is medial to coracobrachialis and lateral to the tensor fasciae antibrachii and overlies a small segment of the medial head of the triceps muscle[27,28].

NOTE: Place a retractor to keep the skin incision open, allowing easier access to the brachial artery. Place a second retractor (optional) to further assist in vessel exposure.

2.3. Moisten all tissues with warm saline (37 °C) for the entire dissection to retain better structural integrity and improved tissue handling during the procedure.

2.4. Create a tunnel under the artery with blunted forceps, then pass three 2-0 polyglactin sutures underneath the artery. Intentionally, leave the ends of this suture relatively long (3–4 cm) to secure the catheter to the artery. Add a “loose suture tie” allowing for quick catheter fastening, to the first two sutures that are separated 1.0 cm from each other and are approximately 1.5–2.0 cm proximal to the third distal suture. Ligate the most distal suture first to occlude the artery.

2.5. Insert a 22 G, 1 inch peripheral venous catheter into the artery and then advance the catheter (completely to the catheter hub) off the stylet into the vessel. Partially withdraw the stylet from the catheter to visualize arterial blood, ensuring proper vessel placement of the catheter. Then, firmly secure the catheter in the vessel by tying the middle suture. Remove the stylet and quickly cap the catheter to minimize bleeding.

2.6. Flush the incision and catheter with warm saline (37 °C). Tie the most proximal suture and importantly ensure that the distal suture is tightly secured around the catheter hub as this improves catheter stability and reduces accidental slippage of the catheter from the artery (i.e., during pig repositioning).

NOTE: If the initial placement of the catheter into the artery fails, or the vessel is injured, reinsert the catheter into the artery at a position approximately 0.25 cm proximal to the initial catheter insertion site.

2.7. Quickly attach the LRS filled intravenous extension line with the connected arterial pressure transducer to the catheter, and then lavage the surgical site with warm saline (37 °C), keeping tissues moist, and clean any blood that spilled into the surrounding tissue. Flush the catheter with saline to ensure catheter patency and prevent blood clots from forming along the catheter wall.

NOTE: Check for transducer arterial blood pressure line failures (i.e., leaks), establish transducer baseline by zeroing arterial pressure monitor measurements, and ensure proper arterial blood pressure wave formations.

2.8. Ensure continued catheter patency by maintaining the flush port of the extension line pressurized above 250 mmHg, with a pressure infuser bag delivering 3–5 mL/min LRS.

2.8.1. Optional: Place either two 2-0 polypropylene or two 2-0 polyglactin sutures around the catheter hub or intravenous extension line hub to further improve catheter stability within the artery.

3. Tissue closure and body positioning

3.1. Close the muscle layers with a simple continuous suture pattern with a 2-0 polyglactin suture on a cutting or tapered needle and close the skin in a simple interrupted suture pattern with a 2-0 polypropylene suture on a cutting needle.

NOTE: Interchangeably, 2-0 polyglactin or 2-0 polypropylene sutures can be used to close muscle and skin.

3.2. Place the pig in ventral recumbency by rotating the abdomen of a lateral recumbent pig toward the surgical table. A left sided lateral recumbent pig is rotated in a clockwise direction, while a right sided lateral recumbent pig is rotated in a counter clockwise direction.

3.3. Place the catheterized forelimb at a 40° angle to the midline of the vertebral column of the pig. This forelimb positioning generates the best arterial blood flow and the most accurate arterial blood pressure measurements.

4. Monitoring clinical parameters

4.1. Measure hemodynamic and respiratory parameters as well as temperature throughout the entire anesthetic and surgical procedure using proper monitoring equipment.

REPRESENTATIVE RESULTS:

Brachial artery catheterization allows for continuous monitoring of arterial blood pressure and intermittent sampling of arterial blood during extended surgical procedures in swine. Measured parameters were collected from seven 50 kg Landrace-Yorkshire commercial pigs as described. The total time required to catheterize the brachial artery was 35.2 ± 4.4 min from the initial artery landmarking to final surgical incision closure (**Figure 1**). The arterial pressures were measured over 120 min and the systolic, diastolic, and mean arterial pressures were 102.9 ± 1.76, 61.2 ± 0.92 and 74.8 ± 0.89 mmHg, respectively (**Figure 2**). Data from **Figure 2** shows a transient drop in systolic blood pressure at 75 min and 120 min during the procedure with subsequent recovery. This is a difficult observation to explain as the event had a short duration and did not appear to have any deleterious effects to the pigs. It is possible the culmination of prolonged surgery, combination of anesthesia (isoflurane gas) and analgesics (remifentanil hydrochloride) affected the systolic blood pressure, but further investigations are needed to determine the mechanism(s) resulting in this observation. Importantly, these measured pressures were similar to arterial pressures observed in pigs anesthetized with either a combination of injectable synthetic narcotic and isoflurane gas or isoflurane gas alone[29-31]. The measured arterial blood gas components over 120 min are shown in **Table 1** and include chemical parameters to evaluate acid base balance, hemoglobin content, and electrolyte concentrations. The values presented were within documented arterial blood pressure and clinical chemistry reference ranges for swine[18,32-34]; however a notable finding was the increased anion gap and reduced total hemoglobin at 120 min. These changes in values were likely associated with physiological changes that can occur during a substantive surgical procedure. Importantly, and in the context of arterial catheterization, the information provided underscores that accurate measures of arterial pressures and arterial blood chemistry are easily obtained from brachial artery catheterization.

FIGURE AND TABLE LEGENDS:

Figure 1: Time requirement: Catheterization of the brachial artery in pigs. Results are expressed as mean ± SEM (*n* = 7). Solid bar = mean and shaded area = SEM

Figure 2: Brachial arterial pressures in pigs. Systolic = systolic arterial pressure; Diastolic = diastolic arterial pressure; MAP = mean arterial pressure. Results are expressed as mean ± SEM (*n* = 7)

Table 1: Brachial arterial blood measurements in pigs. Results are expressed as mean ± SEM (*n* = 7).

DISCUSSION:

Arterial catheterization to measure arterial blood pressures and collect blood samples for arterial blood gas measurements has been established in a wide range of animal species[5-21]. The determination of which artery to choose for catheterization is often directed by either the diagnostic application, experimental protocol, or ease of artery access. Indeed, these factors alter the choice for artery catheterization and are not uniformly utilized across animal species. As an example, median caudal (coccygeal) and pedal arteries have been catheterized in dogs during common surgical procedures in a clinical setting[6,8]. Similarly, the facial and pedal arteries are used in horses to measure arterial pressure due to the relative ease of access in large animal species, such as equids[4,7]. In contrast to dogs, however, median caudal artery catheterization is not a common site used in horses. Considerations for the application of arterial catheterization are also employed to determine the artery of choice to catheterize in swine, although more esoteric arteries have been catheterized for highly specialized procedures[16,17]. In general, four common approaches have been used to measure arterial pressures and collect arterial blood gases in swine. The femoral, carotid and medial saphenous arteries are the most commonly used in large pig techniques, while the auricular artery has been used in piglets and miniature pigs[22,23]. This video describes another method to measure arterial blood pressures and collect arterial blood gas samples in pigs via catheterizing the distal aspect of the brachial artery.

Although catheterization of femoral, carotid, medial saphenous, and auricular arteries enables accurate measurement of arterial pressures and arterial blood gases in swine, there are some potential disadvantages to each technique. Carotid artery catheterization requires deep tissue dissection, with often limited visibility of deep structures and the artery, risking injury to the internal jugular vein, the vagosympathetic nerve trunk, and recurrent nerve[25,35]. The femoral artery is the largest artery of the four common arteries catheterized and should allow for easy catherization[36]. Moreover, due to its relative superficial position, less tissue dissection is required to access the femoral artery compared to the carotid artery. Nonetheless, accessing the artery at the cranial aspect of the inner hind limb or near of the inguinal region for arterial catheterization could limit its use. Indeed, femoral artery catheterization would be an impractical procedure in experiments or surgeries that involve the caudoventral, caudodorsal back, or hind limbs. The medial saphenous is a moderate sized artery and could allow for relatively easy catherization. Similar to the femoral artery, the anatomic location of the medial saphenous artery, as it traverses the medial distal aspect of the hind limb, would limit its application for procedures that involve the lower body of pigs[13]. Auricular catheterization is a fast method to measure arterial pressures due to the superficial position in the ear[11]. A potential limitation with this technique is the difficulty of securing the catheter within the vessel, as there is a limited amount of thick skin within the pig ear to place stay sutures. Another limitation is that the auricular artery is also a small artery, and this could potentially cause more technical difficulties for the catheterization procedure[12] and viability during long research procedures. More importantly, the greatest disadvantage of catheterizing the auricular artery is the presence of marginal ear veins[11,12] that are often the primary vessels used to provide intravenous fluids and administer drugs for anesthesia during surgical procedures. The close proximity of the auricular artery and marginal ear veins, makes the simultaneous catheterization of the vein and artery technically challenging and could interfere with both arterial blood measurements and the administration of drugs and fluids. Catheterization of the distal brachial artery overcomes some of these disadvantages. Firstly, isolation of the brachial artery requires less tissue dissection than isolation of the carotid artery. The brachial artery is also anatomically located closer to the surface, allowing for an easier catheterization, compared to the deeper carotid artery. Secondly, catheterization of brachial artery is a relatively quick procedure and although not as fast as the auricular artery, placement within this vessel permits unrestricted access to marginal ear veins; an important requirement for rehydration therapy and the administration of intravenous drugs and anesthetics during surgery. Finally, a significant benefit of distal brachial arterial catheterization, compared to femoral and medial saphenous artery catherization, is that the procedure does not interfere with surgical or experimental procedures involving caudoventral, and caudodorsal back, or the hind limbs.

As with all experimental methods there are critical steps needed for the successful outcome of procedure. There are three important processes that require particular attention during brachial arterial catheterization. First, accurate topographical landmarking by palpation of the faint pulse of the distal brachial artery is needed. Deviation of the proper location of the vessel within the forelimb will alter the direction of tissue dissection to isolate the vessel and cause unnecessary tissue dissection and injury. Second, careful tissue dissection and catheter insertion is required. This includes gentle separation of the artery from the surrounding fascia, the juxtaposed brachial vein, and the median nerve (minimizing injury to the median nerve), as well as the insertion of the catheter into the vessel with the addition of stay ligatures to secure the catheter into the vessel. Notably, although the brachial artery is a medium sized robust vessel[17], rough handling of the artery can lead to the development of microhemorrhages within the vessel intima and potentially induce clot formation along the damaged vessel, thus, increasing the chance of catheterization failure[37]. Finally, proper positional alignment of the catheterized forelimb is essential for accurate and consistent arterial pressure measurements. Specifically, extending the forelimb at a 40° angle from the midline of the vertebral column provided the best results. Placing the catheterized limb mid thorax and alongside the body, or extending the forelimb directly forward and parallel to the midline of the back may cause a modest reduction in arterial pressures and can be more prone to intermittent loss of arterial pressure measurements.

All surgical procedures have innate limitations and the catherization of the distal brachial artery is no exception. Identifying, localizing, isolating, and catheterizing the artery is a procedure that requires individuals to have a good understanding of not only topographical and vascular anatomy, but also excellent technical and surgical skills. People with this knowledge base and skill set will likely be more successful with catheterizing the brachial artery than people with limited previous experience. The most substantive limitation of this procedure is that brachial artery catheterization may be restricted to non-recovery experimental procedures. Applying this technique to a recovery procedure would require either the removal of the distal suture securing the catheter to the vessel or omitting placing the distal suture during the procedure with a potential subsequent loss of stability. Additionally, in order to secure the catheter hub on the outside of the sutured muscle and skin, a longer catheter would be required. Finally, a recovery procedure would require the removal of the catheter from the vessel. This activity could lead to marked hemorrhaging if adequate hemostasis was not obtained; a process that could be particularly difficult for relatively deep vessels of the limbs of large animal species. Furthermore, if adequate hemostasis was achieved, it is possible the newly formed clot could dislodge during movement or from minor impact (i.e. against pen wall), leading to profound and life-threating arterial hemorrhaging.

In conclusion, brachial arterial catheterization is an excellent method to measure arterial blood pressures and collect arterial blood for blood measurements. This method produced results in line with observations of arterial blood parameter measurements from the catheterization of different arteries in the pig. In swine this procedure is particularly useful in non-recovery experimental procedures that involve the caudoventral, caudodorsal back, and hind limbs of the pig.

ACKNOWLEDGMENTS:

This work was supported by the Canadian Institutes of Health Research and Canada Foundation for Innovation grant to V. K. Mushahwar. A. Toossi was supported by a Vanier Canada Graduate Scholarship, Alberta Innovates – Health Solutions Graduate Studentship and a Queen Elizabeth II Graduate Scholarship. We would like to acknowledge Mr. J. Stack of Moss Street Productions for his help with the audio production and staff of the Surgical Medical Research Institute for their assistance with the procedures.

DISCLOSURES:

The authors have nothing to disclose.

REFERENCES

1 Uwiera, R. R. et al. Plasmid DNA induces increased lymphocyte trafficking: a specific role for CpG motifs. *Cellular Immunology.* **214** (2), 155-164, doi:10.1006/cimm.2001.1899 (2001).

2 Uwiera, R. R., Kastelic, J. P., Inglis, G. D. Catheterization of intestinal loops in ruminants. *Journal of Visualized Experiments.* (28), doi:10.3791/1301 (2009).

3 Uwiera, R. R., Mangat, R., Kelly, S., Uwiera, T. C., Proctor, S. D. Long-Term Catheterization of the Intestinal Lymph Trunk and Collection of Lymph in Neonatal Pigs. *Journal of Visualized Experiments.* (109), doi:10.3791/53457 (2016).

4 Wohlfender, D. H. et al. International online survey to assess current practice in equine anaesthesia. *Equine Veterinary Journal.* **47**, 65-71, doi:10.1111/evj.12257 (2015).

5 Dugdale, A. H., Taylor, P. M. Equine anaesthesia-associated mortality: where are we now? *Veterinay Anaesthesia Analgesia.* **43** (3), 242-255, doi:10.1111/vaa.12372 (2016).

6 McGrotty, Y., Brown, A. Blood gases, electrolytes and interpretation 1. Blood gases. *In Practice.* **35** (2), 59-65, doi:10.1136/inp.f569 (2013).

7 Taylor, P. M. Techniques and clinical application of arterial blood pressure measurement in the horse. *Equine Veterinary Journal.* **13**, 271-275 (1981).

8 Trim, C. M., Hofmeister, E. H., Quandt, J. E., Shepard, M. K. A survey of the use of arterial catheters in anesthetized dogs and cats: 267 cases. *Journal of Veterinary Emergency and Critical Care.* **27**, 89-95, doi:10.1111/vec.12543 (2017).

9 Komine, H., Matsukawa, K., Tsuchimochi, H., Nakamoto, T., Murata, J. Sympathetic cholinergic nerve contributes to increased muscle blood flow at the onset of voluntary static exercise in conscious cats. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology.* **295** (4), R1251-1262, doi:10.1152/ajpregu.00076.2008 (2008).

10 Krista, L. M., Beckett, S. D., Branch, C. E., McDaniel, G. R., Patterson, R. M. Cardiovascular Responses in Turkeys as Affected by Diurnal Variation and Stressor. *Poultry Science.* (60), 462-468 (1981).

11 Bass, L. M., Yu, D. Y., Cullen, L. K. Comparison of femoral and auricular arterial blood pressure monitoring in pigs. *Veterinay Anaesthesia Analgesia.* **36** (5), 457-463, doi:10.1111/j.1467-2995.2009.00484.x (2009).

12 Karnabatidis, D., Katsanos, K., Diamantopoulous, A., Kagadis, G. C.,, Siablis, D. Transauricular arterial or venous access for cardiovascular experimental protocols in animals. *Journal of Vascular and Interventional Radiology*. **17** (11 Pt 1), 1803-1811, doi:10.1097/01.RVI.0000244836.16098.B1 (2006).

13 Namba, K., Kawamura, Y., Higaki, A., Nemoto, S. Percutaneous medial saphenous artery approach for Swine central artery access. *Journal of Investigative Surgery.* **26** (6), 360-363, doi:10.3109/08941939.2013.797058 (2013).

14 Hong, Y. et al. Feasibility of Selective Catheter-Directed Coronary Computed Tomography Angiography Using Ultralow-Dose Intracoronary Contrast Injection in a Swine Model. *Investigative Radiology.* (50), 449-455 (2015).

15 Kumar, A. et al. Aortic root catheter-directed coronary CT angiography in swine: coronary enhancement with minimum volume of iodinated contrast material. *American Journal of Roentgenology.* (188), W415-422, doi:10.2214/AJR.06.0945 (2007).

16 Park, J. H. et al. Safety and Efficacy of an Aortic Arch Stent Graft with Window-Shaped Fenestration for Supra-Aortic Arch Vessels: an Experimental Study in Swine. *Korean Circulation Journal.* **47** (2), 215-221, doi:10.4070/kcj.2016.0286 (2017).

17 Carniato, S., Mehra, M., King, R. M., Wakhloo, A. K., Gounis, M. J. Porcine brachial artery tortuosity for in vivo evaluation of neuroendovascular devices. *American Journal of Neuroradiology.* **34** (4), E36-38, doi:10.3174/ajnr.A2778 (2013).

18 Hannon, J. P., Bossone, C. A., Wade, C. E. Normal Physiological Values for Consious pigs used in Biomedical Research. *Laboratory Animal Science.* **40**, 293-298 (1990).

19 Nijland, M. J., Shankar, U., Iyer, V., Ross, M. G. Assessment of fetal scalp oxygen saturation determination in the sheep by transmission pulse oximetry. *American Journal of Obstetrics and Gynecology.* **183** (6), 1549-1553, doi:10.1067/mob.2000.107736 (2000).

20 Yawno, T. et al. Human Amnion Epithelial Cells Protect Against White Matter Brain Injury After Repeated Endotoxin Exposure in the Preterm Ovine Fetus. *Cell Transplantation.* **26**

(4), 541-553, doi:10.3727/096368916X693572 (2017).
21 Amaya, K. E. et al. Accelerated acidosis in response to variable fetal heart rate decelerations in chronically hypoxic ovine fetuses. *American Journal of Obstetrics and Gynecology.* **214** (2), 270 e271-270 e278, doi:10.1016/j.ajog.2015.09.084 (2016).
22 Malavasi, L. M. Swine. *Anesthesia and Analgesia for Domestic Species*. 5 edn, 936 John Wiley & Sons Inc. New Jersey (2015).
23 Moon, P. F., Smith, L. J. General Anesthetic Techniques in Swine. *Veterinary Clinics of North America: Food Animal Practice.* **12** (3), 663-691, doi:10.1016/s0749-0720(15)30392-3 (1996).
24 Caramoni, P. R. A. et al. Postangioplasty restenosis: a practical model in the porcine carotid artery. *Brazilian Journal of Medical and Biological Research.* (30), 1087-1091.
25 Sisson, S. *The Anatomy of the Domestic Animals*. 2 edn, Vol. 1 636-638, W. B. Saunders Company. Philadelphia (1930).
26 Lundeed, A. B., Manohar, M., Parks, C. Systemic distribution of blood flow in swine while awake and during 1.0 and 1.5 MAC isoflurane anesthesia with or without 50% nitrous oxide. *Anesthesia and Analgesia.* **31**, 499-512 (1983).
27 Sisson, S. *The Anatomy of the Domestic Animals.* 2 edn, Vol. 1 302-304, W. B. Saunders Company. Philadelphia (1930).
28 Adin, C. A., Gregory, C. R., Adin, D. B., Cowgill, L. D., Kyles, A. E. Evaluation of three peripheral arteriovenous fistulas for hemodialysis access in dogs. *Veterinary Surgery.* **31** (5), 405-411, doi:10.1053/jvet.2002.34663 (2002).
29 Gladczak, A. K., Shires, P. K., Stevens, K. A., Clymer, J. W. Comparison of indirect and direct blood pressure monitoring in normotensive swine. *Research in Veterinary Science.* **95** (2), 699-702, doi:10.1016/j.rvsc.2013.05.013 (2013).
30 Wenzel, K. et al. Survey of Effects of Anesthesia Protocols on Hemodynamic Variables in Porcine Cardiopulmonary Resuscitation Laboratory Models Before Induction of Cardiac Arrest. *Comparative Medicine* **50** (6), 644-648 (2000).
31 Duval, J. D., Pang, J. M., Boysen, S. R., Caulkett, N. A. Cardiopulmonary Effects of a Partial Intravenous Anesthesia Technique for Laboratory Swine. *Journal of the American Association for Laboratory Animal Science.* **57** (4), 376-381, doi:10.30802/AALAS-JAALAS-17-000164 (2018).
32 Friendship, R. M., Lumsden, J. H., McMillan, I., Wilson, M. R. Hematology and Biochemistry Reference Values for Ontario Swine. *Canadian Journal of Comparative Medicine.* (48), 390-393 (1984).
33 Kiorpes, A. L., MacWilliams, P. S., Schenkman, D. I., Bickstrom, L. R. Blood Gas and Hematological Changes in Experimental Peracute Porcine Pleuropneumonia. *Canadian Journal of Veterinary Research.* (54), 164-169 (1990).
34 Klem, T. B., Bleken, E., Morberg, H., Thoresen, S. I., Framstad, T. Hematologic and biochemical reference intervals for Norwegian crossbreed grower pigs. *Veterinary Clinical Pathology.* **39** (2), 221-226, doi:10.1111/j.1939-165X.2009.00199.x (2010).
35 Sisson, S. *The Anatomy of the Domestic Animals*. 2 edn, Vol. 1 804-810, W. B. Saunders Company. Philadelphia (1930).
36 Lopes-Berkas, V. C., Jorgenso, M. A. Measurement of Peripheral Arterial Vasculature in Domestic Yorkshire Swine by Using Quantitative Vascular Angiograph. *Journal of the*

American Association for Laboratory Animal Science. **50** (5), 628-634 (2011).
37 Geary, R. L. et al. Wound healing: A paradigm for lumen narrowing after arterial reconstruction. *Journal of Vascular Surgery.* (27), 96-108 (1998).

Figure 1

Click here to access/download;Figure;Figure 1 revision.tif

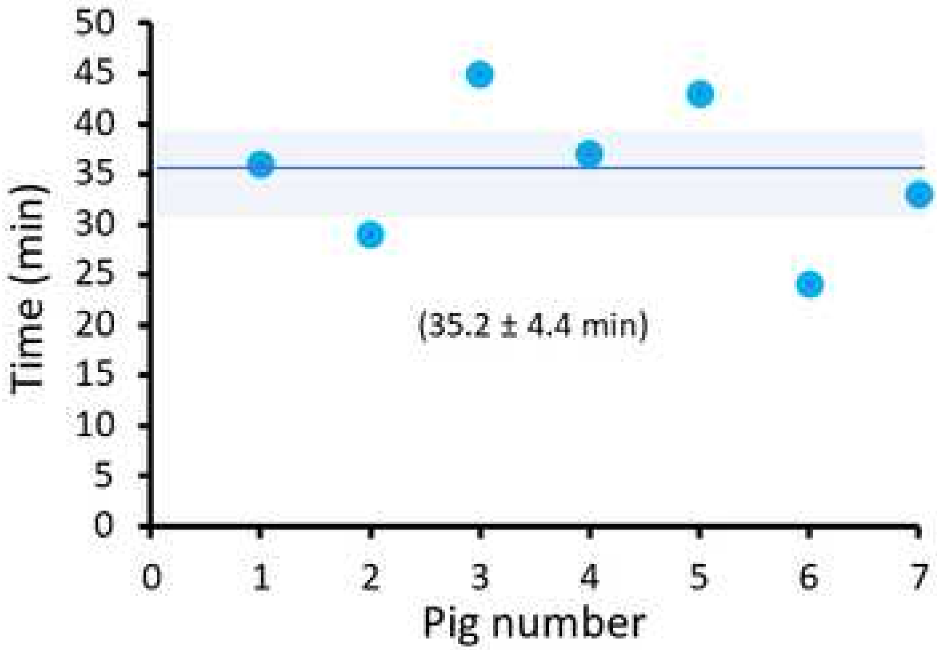
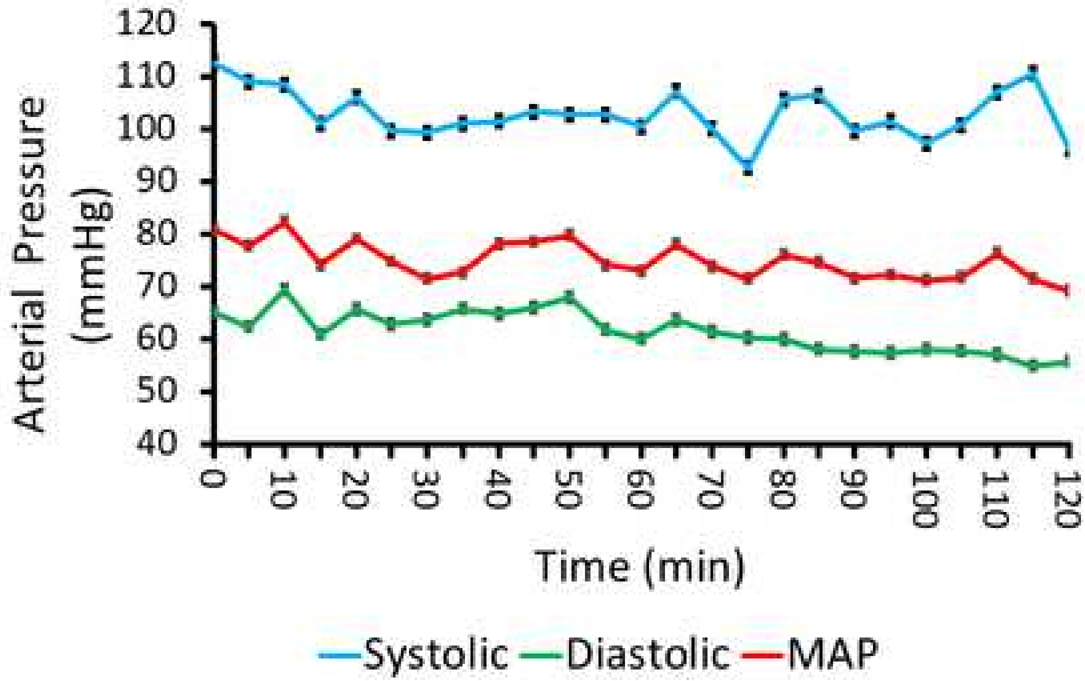


Figure 2

Click here to access/download;Figure;Figure 2 revision.tif



Time (min)	pH	HCO_3 (mmol/L)	AnGap (mmol/L)	Base Excess (mmol/L)	PCO_2 (mmHg)	Total Hb (g/dL)	Na^+ (mmol/L)	K^+ (mmol/L)
0	7.50 ± 0.03	24.8 ± 1.9	11.5 ± 1.1	3.0 ± 1.7	34.3 ± 3.2	13.1 ± 1.1	141.0 ± 1.1	4.5 ± 0.5
120	7.47± 0.03	25.0±2.7	14.0 ±1.2	2.0 ± 1.3	34.0 ± 3.9	10.3 ± 0.8	139.4 ± 0.6	5.0 ± 0.4

Cl^- (mmol/L)
109.2 ± 1.5
107.5 ± 0.6

Name of Material/ Equipment	Company	Catalog Number	Comments/Description/Quantity
0.9% NaCl (Saline) Solution	EMRN	JB1322P	1 x1 liter bag
10% Lidocaine spray	AstraZeneca		DIN:02039508 / 1 x 50 ml bottle
10% Povidone-Iodine scrub	Purdue Pharma	521232	1 x 500 ml bottle
20 ga 1-inch angiocatheter	Becton Dickinson	381433	1 x angiocatheter
2-0 polyglactin suture (Vicryl)	Ethicon	J339H	2-0 vicryl / 1 packet of suture
2-0 polypropylene suture (Prolene)	Ethicon	8833H	2-0 prolene / 1 packet of suture
22 ga 1-inch angiocatheter	Becton Dickinson	381423	1 x angiocatheter
9 ID mm endotracheal tube	Jorvet	J0835P	1 x endotracheal tube
Arterial blood pressure IV line	Argon Medical Devices	112411	1 x arterial blood pressure IV line
Disposable drapes	Halyard Sales LLC	89731	4-8 x disposable drapes
Glycopyrrolate hydrochloride	Sandoz		DIN:02039508 / 1 x 20ml vial
Isoflurane	Abbott Animal Health	05260-5	1 x 250ml bottle
Kelly forceps-curved (14cm)	Stevens	162-7-38	8-10 instruments
Ketamine hydrochloride	Vetoquinol		DIN:02374994 / 1 x 10ml vial
Lactated Ringer's Solution	Hospira	0409-7953-09	4 x1 liter bag
Metzenbaum scissors	Fine Science	14518-18	
Miller laryngoscope blade	Welch Allyn	68044	182 mm length / 1 instrument
Nasal temperature probe	Surgivet	V3417	1 probe
Needle Drivers	Stevens	162-V98-42	2 instruments
Q tip applicators	Fisher Scientific	22-037-960	20-40 app
Remifentanil hydrochloride	TEVA		DIN:0234432 / 1 mg vial
Surgivet advisor: Vital signs monitor	Surgivet	V9203	1 monitor
Weitlaner retractor	Stevens	162-11-602	2 retractors
Xylazine hydrochloride	Bayer		DIN:02169606 1 x 50ml bottle

JoVE JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

Title of Article: Brachial Artery Catheterization in Swine

Author(s): Uwiera, RRE, Toossi A, Everaert DG, Uwiera TC, Mushahwar VK

Item 1 (check one box): The Author elects to have the Materials be made available (as described at http://www.jove.com/author) via: [x] Standard Access [ ] Open Access

Item 2 (check one box):

[x] The Author is NOT a United States government employee.

[ ] The Author is a United States government employee and the Materials were prepared in the course of his or her duties as a United States government employee.

[ ] The Author is a United States government employee but the Materials were NOT prepared in the course of his or her duties as a United States government employee.

ARTICLE AND VIDEO LICENSE AGREEMENT

1. Defined Terms. As used in this Article and Video License Agreement, the following terms shall have the following meanings: "**Agreement**" means this Article and Video License Agreement; "**Article**" means the article specified on the last page of this Agreement, including any associated materials such as texts, figures, tables, artwork, abstracts, or summaries contained therein; "**Author**" means the author who is a signatory to this Agreement; "**Collective Work**" means a work, such as a periodical issue, anthology or encyclopedia, in which the Materials in their entirety in unmodified form, along with a number of other contributions, constituting separate and independent works in themselves, are assembled into a collective whole; "**CRC License**" means the Creative Commons Attribution-Non Commercial-No Derivs 3.0 Unported Agreement, the terms and conditions of which can be found at: http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode; "**Derivative Work**" means a work based upon the Materials or upon the Materials and other pre-existing works, such as a translation, musical arrangement, dramatization, fictionalization, motion picture version, sound recording, art reproduction, abridgment, condensation, or any other form in which the Materials may be recast, transformed, or adapted; "**Institution**" means the institution, listed on the last page of this Agreement, by which the Author was employed at the time of the creation of the Materials; "**JoVE**" means MyJove Corporation, a Massachusetts corporation and the publisher of *The Journal of Visualized Experiments;* "**Materials**" means the Article and / or the Video; "**Parties**" means the Author and JoVE; "**Video**" means any video(s) made by the Author, alone or in conjunction with any other parties, or by JoVE or its affiliates or agents, individually or in collaboration with the Author or any other parties, incorporating all or any portion of the Article, and in which the Author may or may not appear.

2. Background. The Author, who is the author of the Article, in order to ensure the dissemination and protection of the Article, desires to have the JoVE publish the Article and create and transmit videos based on the Article. In furtherance of such goals, the Parties desire to memorialize in this Agreement the respective rights of each Party in and to the Article and the Video.

3. Grant of Rights in Article. In consideration of JoVE agreeing to publish the Article, the Author hereby grants to JoVE, subject to **Sections 4** and **7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Article in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Article into other languages, create adaptations, summaries or extracts of the Article or other Derivative Works (including, without limitation, the Video) or Collective Works based on all or any portion of the Article and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. If the "Open Access" box has been checked in **Item 1** above, JoVE and the Author hereby grant to the public all such rights in the Article as provided in, but subject to all limitations and requirements set forth in, the CRC License.

4. Retention of Rights in Article. Notwithstanding the exclusive license granted to JoVE in **Section 3** above, the Author shall, with respect to the Article, retain the non-exclusive right to use all or part of the Article for the non-commercial purpose of giving lectures, presentations or teaching classes, and to post a copy of the Article on the Institution's website or the Author's personal website, in each case provided that a link to the Article on the JoVE website is provided and notice of JoVE's copyright in the Article is included. All non-copyright intellectual property rights in and to the Article, such as patent rights, shall remain with the Author.

5. Grant of Rights in Video – Standard Access. This **Section 5** applies if the "Standard Access" box has been checked in **Item 1** above or if no box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby acknowledges and agrees that, Subject to **Section 7** below, JoVE is and shall be the sole and exclusive owner of all rights of any nature, including, without limitation, all copyrights, in and to the Video. To the extent that, by law, the Author is deemed, now or at any time in the future, to have any rights of any nature in or to the Video, the Author hereby disclaims all such rights and transfers all such rights to JoVE.

6. Grant of Rights in Video – Open Access. This **Section 6** applies only if the "Open Access" box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby grants to JoVE, subject to **Section 7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Video in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Video into other languages, create adaptations, summaries or extracts of the Video or other Derivative Works or Collective Works based on all or any portion of the Video and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. For any Video to which this Section 6 is applicable, JoVE and the Author hereby grant to the public all such rights in the Video as provided in, but subject to all limitations and requirements set forth in, the CRC License.

7. Government Employees. If the Author is a United States government employee and the Article was prepared in the course of his or her duties as a United States government employee, as indicated in **Item 2** above, and any of the licenses or grants granted by the Author hereunder exceed the scope of the 17 U.S.C. 403, then the rights granted hereunder shall be limited to the maximum rights permitted under such statute. In such case, all provisions contained herein that are not in conflict with such statute shall remain in full force and effect, and all provisions contained herein that do so conflict shall be deemed to be amended so as to provide to JoVE the maximum rights permissible within such statute.

8. Likeness, Privacy, Personality. The Author hereby grants JoVE the right to use the Author's name, voice, likeness, picture, photograph, image, biography and performance in any way, commercial or otherwise, in connection with the Materials and the sale, promotion and distribution thereof. The Author hereby waives any and all rights he or she may have, relating to his or her appearance in the Video or otherwise relating to the Materials, under all applicable privacy, likeness, personality or similar laws.

9. Author Warranties. The Author represents and warrants that the Article is original, that it has not been published, that the copyright interest is owned by the Author (or, if more than one author is listed at the beginning of this Agreement, by such authors collectively) and has not been assigned, licensed, or otherwise transferred to any other party. The Author represents and warrants that the author(s) listed at the top of this Agreement are the only authors of the Materials. If more than one author is listed at the top of this Agreement and if any such author has not entered into a separate Article and Video License Agreement with JoVE relating to the Materials, the Author represents and warrants that the Author has been authorized by each of the other such authors to execute this Agreement on his or her behalf and to bind him or her with respect to the terms of this Agreement as if each of them had been a party hereto as an Author. The Author warrants that the use, reproduction, distribution, public or private performance or display, and/or modification of all or any portion of the Materials does not and will not violate, infringe and/or misappropriate the patent, trademark, intellectual property or other rights of any third party. The Author represents and warrants that it has and will continue to comply with all government, institutional and other regulations, including, without limitation all institutional, laboratory, hospital, ethical, human and animal treatment, privacy, and all other rules, regulations, laws, procedures or guidelines, applicable to the Materials, and that all research involving human and animal subjects has been approved by the Author's relevant institutional review board.

10. JoVE Discretion. If the Author requests the assistance of JoVE in producing the Video in the Author's facility, the Author shall ensure that the presence of JoVE employees, agents or independent contractors is in accordance with the relevant regulations of the Author's institution. If more than one author is listed at the beginning of this Agreement, JoVE may, in its sole discretion, elect not take any action with respect to the Article until such time as it has received complete, executed Article and Video License Agreements from each such author. JoVE reserves the right, in its absolute and sole discretion and without giving any reason therefore, to accept or decline any work submitted to JoVE. JoVE and its employees, agents and independent contractors shall have

full, unfettered access to the facilities of the Author or of the Author's institution as necessary to make the Video, whether actually published or not. JoVE has sole discretion as to the method of making and publishing the Materials, including, without limitation, to all decisions regarding editing, lighting, filming, timing of publication, if any, length, quality, content and the like.

11. Indemnification. The Author agrees to indemnify JoVE and/or its successors and assigns from and against any and all claims, costs, and expenses, including attorney's fees, arising out of any breach of any warranty or other representations contained herein. The Author further agrees to indemnify and hold harmless JoVE from and against any and all claims, costs, and expenses, including attorney's fees, resulting from the breach by the Author of any representation or warranty contained herein or from allegations or instances of violation of intellectual property rights, damage to the Author's or the Author's institution's facilities, fraud, libel, defamation, research, equipment, experiments, property damage, personal injury, violations of institutional, laboratory, hospital, ethical, human and animal treatment, privacy or other rules, regulations, laws, procedures or guidelines, liabilities and other losses or damages related in any way to the submission of work to JoVE, making of videos by JoVE, or publication in *JoVE* or elsewhere by JoVE. The Author shall be responsible for, and shall hold JoVE harmless from, damages caused by lack of sterilization, lack of cleanliness or by contamination due to the making of a video by JoVE its employees, agents or independent contractors. All sterilization, cleanliness or decontamination procedures shall be solely the responsibility of the Author and shall be undertaken at the Author's expense. All indemnifications provided herein shall include JoVE's attorney's fees and costs related to said losses or damages. Such indemnification and holding harmless shall include such losses or damages incurred by, or in connection with, acts or omissions of JoVE, its employees, agents or independent contractors.

12. Fees. To cover the cost incurred for publication, JoVE must receive payment before production and publication the Materials. Payment is due in 21 days of invoice. Should the Materials not be published due to an editorial or production decision, these funds will be returned to the Author. Withdrawal by the Author of any submitted Materials after final peer review approval will result in a US$1,200 fee to cover pre-production expenses incurred by JoVE. If payment is not received by the completion of filming, production and publication of the Materials will be suspended until payment is received.

13. Transfer, Governing Law. This Agreement may be assigned by JoVE and shall inure to the benefits of any of JoVE's successors and assignees. This Agreement shall be governed and construed by the internal laws of the Commonwealth of Massachusetts without giving effect to any conflict of law provision thereunder. This Agreement may be executed in counterparts, each of which shall be deemed an original, but all of which together shall be deemed to me one and the same agreement. A signed copy of this Agreement delivered by facsimile, e-mail or other means of electronic transmission shall be deemed to have the same legal effect as delivery of an original signed copy of this Agreement.

A signed copy of this document must be sent with all new submissions. Only one Agreement required per submission.

CORRESPONDING AUTHOR:

Name: Uwiera, Richard R. E

Department: Department of Agricultural Food and Nutritional Science

Institution: University of Alberta

Article Title: Brachial Artery Catheterization in Swine

Signature: Richard Uwiera Date: Nov 5, 2018

Please submit a signed and dated copy of this license by one of the following three methods:

1) Upload a scanned copy of the document as a pfd on the JoVE submission site;
2) Fax the document to +1.866.381.2236;
3) Mail the document to JoVE / Attn: JoVE Editorial / 1 Alewife Center #200 / Cambridge, MA 02139

For questions, please email submissions@jove.com or call +1.617.945.9051

February 10, 2019

Dear JOVE

RE: Uwiera et al: Responses to editorial, reviewers' and veterinarian comments

Please find our responses to the comments that were provide by the reviewers, editors, production editors and the veterinarian. The authors feel we have adequately, and to the best of our abilities, addressed all comments.

1) Editorial and production comments:

Changes to be made by the author(s) regarding the manuscript:
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

Author's response: The required editorial changes and further proofreading were completed.

2. Please use SI abbreviations for time units: h, min, s.

Author's response: The required editorial and productions changes were completed.

3. Please revise the protocol to contain only action items that direct the reader to do something (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "Note." Please include all safety procedures and use of hoods, etc. However, notes should be used sparingly and actions should be described in the imperative tense wherever possible. Please move the discussion about the protocol to the Discussion.

Author's response: The required editorial and productions changes were completed.

4. Please revise the Protocol steps so that individual steps contain only 2-3 actions per step and a maximum of 4 sentences per step. Use sub-steps as necessary

Author's response: The required formatting protocol were completed.

5. References: Please do not abbreviate journal titles.

Author's response: The journal titles were adjusted to provide the full name of the journal.

6. Table of Equipment and Materials: Please sort the items in alphabetical order according to the name of material/equipment.

<u>Author's response</u>: The required editorial changes to "Equipment and Materials" were completed

Changes to be made by the Author(s) regarding the video:
1. Please increase the homogeneity between the written protocol and the narration in the video. It would be best if the narration is a word for word from the written protocol text.

<u>Author's response</u>: The narrative and text have been adjusted (to the best of our abilities) in several areas so there is an improvement in homogeneity between the video and written manuscript. The authors ensured (to the best of our abilities) that the video narrative and manuscript text did not appear asynchronous.

2. The video must have chapter title cards (Introduction, Protocol, Representative Results, and Conclusions).

<u>Author's response</u>: The required video edits were completed.

3. Please ensure that the section title cards used in the video are the same as the section headings used in the written protocol.

<u>Author's response</u>: The required video edits were completed.

4. 1:16: Please mention in the video how large the incision is.

<u>Author's response</u>: A statement indicating a 6cm incision length was included in the video.

5. 2:00-2:12, 4:11-4:12: Such details in the video are not mentioned in the written protocol.

<u>Author's response</u>: Video (2:00-2:12)- We have added a statement in the text (lines 175-177) regarding the close proximity of the structures to more closely align with the video segment. Video (4:11-4:12)- We added a statement regarding flushing the incision and catheter with warm saline (line 199)

6. Most of the edits in this video are fades to black. This causes the viewer to have to readjust their focus for every edit. We recommend using simple crossfade edits instead.

<u>Author's response</u>: The video removed fade to black and added crossfade edits

7. It appears that most of the live-action video has been sped up. Especially because this is an animal surgery, the action should not be sped up.

<u>Author's response</u>: The video clip speed has not been altered within the production, and all video clips are in real time. As such no adjustments or edits can be made.

8. 3:16 - The word "vessel" sounds cut off. This should be fixed.

<u>Author's response</u>: The required video edit is completed.

9. Please upload a revised high-resolution video here:
https://www.dropbox.com/s/jxui0sw26ur4dm3/Uwiera%20BACS%20Nov%202018%20Final.wmv?dl=0

2) Reviewers' comments:

Reviewer #1:
Manuscript Summary:
Uwiera and colleagues describe a method for catheterization of the brachial artery in swine. This method is an alternative for more conventional vascular access points (i.e. femoral, carotid, etc.) to collect blood for arterial blood gases (ABG) and monitor arterial blood pressure during surgical procedures that require the animal in a caudoventral, caudodorsal positioning. The manuscript is well written and articulated; the sections within the protocol include: premedication, surgical prep, anatomical demarcation of the brachial artery, catheterization and representative results. The figures demonstrate nicely the time of the procedure and results from ABG and arterial blood pressure. If the authors address a few corrections and minor changes it would enhance the manuscript/video.

Concerns:
Written Manuscript
1. Using isoflurane for general anesthesia is standard, however isoflurane can have negative effects of central and systemic hemodynamics. The authors have countered this through the infusion of LRS. A note under section 1.3 would acknowledge the effects of isoflurane on hemodynamics for those reading the manuscript.

<u>Author's response</u>: A statement indicating the depressive cardiovascular effects of isoflurane and subsequent LRS infusion to support cardiovascular output was added in the protocol section 1.4.1 (lines 138-140).

2. The addition of a written section discussing the pressure transducer setup and positioning to the animal may also enhance the manuscript. While the focus is on catheterization of the artery, the authors discuss the utility of this access for the ABG and blood pressure measurements. Some description of proper setup, zeroing and normal waveform measurements would be nice

<u>Author's response</u>: Statements regarding positioning of the transducer relative to the pig's body position (section 1.2; lines 123-126) and establishing baseline (section 2.7.1; lines 212-214) has been added to the manuscript.

3. Figure 1 illustrates the time of the procedures for seven animals. Could the authors put a line at the mean and dotted lines at the +/- the standard error. This would give readers a quick and easier visualization of this figure along with the raw data points.

Author's response: Adjustments were made in line with the reviewer's request. Figure 1-A solid line representing mean and a shaded area representing SEM was added to Figure 1. Following several graphic variations, the author's believed the shaded area facilitate easier demarcation of SEM compared to dotted line. Figure 1 legend was also adjusted to reflect changes to the Figure (line 273).

4. Figure 2 illustrates arterial blood pressure over 120 minutes. The figure legend says that this is the average of seven animals +/- the standard error of their diastolic, systolic and mean pressure. For example, at 15 minutes, systolic pressure is 100, diastolic is 60, mean pressure should be 80 mmHg. However, the graph shows a mean pressure of ~70 mmHg. Can the authors make corrections to mean pressures.

Author's response: The authors thank the reviewer for the comment. Interestingly, mean arterial pressure is measured as: MAP = 1/3 systolic pressure + 2/3 diastolic pressure and is not an equal average between systolic and diastolic pressure- which seems as the most intuitive form for the calculation (Ref- Iaizzo editor; Handbook of cardiac physiology, anatomy and devices). As well, all measurements were provided by the 'Surgivet advisor- Vital signs monitor' as the machine calculates the mean arterial pressure. Moreover, the data was initially double checked between manual arithmetic calculations and monitor MAP values to ensure accuracy of the data. Therefore, the authors feel the values presented in Figure 2 are correct and no adjustments were made.

5. Is the data displayed in figure 2 a single animals' measurement rather than an average? If so, please add the mean data or please add the standard errors to each timepoint. Changing the graphs y-axis scale might show the standard error better.

Author's response: The values in Figure 2 represent the mean and SEM for 7 pigs. The error bars are very small (black lines) and within the thickness line (mean). We have adjusted the y-axis to improve the visualization of the error bars. Although modest, the authors feel there is an enhancement of the graph with corresponding errors bars.

6. In figure 2, it is interesting to see the variability in systolic pressure (i.e. 65-75 min. ~20 mmHg). Could the authors please explain these types of changes in the blood pressure data. Is this due to surgical procedure, outliers, changes in anesthesia or fluids?

Author's response: A statement addressing the changes in systolic blood pressure was added to the manuscript (lines 251-257).

7. Table 1, shows baseline and final ABG measurements. Were measurements taken at time intervals between baseline and final? Please include ABG every 30 min.

Author's response: Thank you for the reviewer's comment. Unfortunately, in this experiment blood gas measurements were only collected at 0 min and 120 min and as such we are unable to provide more *blood gas data. The experimental procedure (spinal surgeries) are completed and new experiments have not been planned and any subsequent surgeries would will not occur for several years. It would be particularly challenging to obtain ICUC (ACUC) approval for brachial arterial catherization without the spinal surgery – the primary focus of the experiment.*

Video:
1. Adding a section at the beginning of the video regarding sterile preparation of the surgical site would enhance video.

<u>Author's response</u>: A video clip demonstrating the application of surgical scrub to the incision site was added. A video demonstrating the hair removal (clipping) was not obtained during the procedure and could not be added to video production.

2. At 3:05 - 3:11 minutes, please identify the distal and proximal suture either verbally or with some other annotation.

<u>Author's response</u>: The identification of the proximal and distal sutures has been added to the video.

3. At 3:17 section entitled "Brachial Artery Catheterization & Securing Catheter with Suture" camera angle is to narrow, should be shot with a wider angle, so that viewer can visualize entire field where cannulation, securing suture and connect pressure line is occurring.

<u>Author's response</u>: Similar to the comment above (Reviewer 1-comment 7)- Unfortunately, the authors cannot obtain any new video footage or camera angles to better the video presentation as the experimental procedure is completed.

4. Could the authors briefly show pressure measurement setup and real time pressure readings at the end of the video prior to figures.

<u>Author's response:</u> Active video for set up and monitoring was not acquired during the procedure. We have however, provided a still image highlighting clinical paraments monitored through the procedure. A corresponding statement was added to the manuscript (protocol section 4.0; subsection4.1, lines 239-242).

Minor Concerns:
1. Line 343 - 344, did the authors mean to say "recovery" procedure? If so, please make correction.

<u>Author's response</u>: The sentence was adjusted to "'recovery" procedure (Line 367). Note the line number has changed due to edits added prior to this statement.

2. Table of Materials, is 10% lidocaine spray supposed to be 2% (as written in the manuscript). Please change if needed to be corrected.

Author's response: A 10% lidocaine spray was used for the procedure and this adjustment was added to the text of the manuscript (Line 130).

3. Please add reasonable quantity of each material on the materials list (i.e. isoflurane - 1 bottle, or Weitlaner retractor – 2

Author's response: The information was added and edits completed.

Reviewer #2:
Manuscript Summary:
This manuscript and video provide a guide to surgical catheterization of the brachial artery in swine. I am not aware of any other detailed description of this technique.

Major Concerns:
None

Minor Concerns:
They recommend that it be done for non survival surgeries due to potential homeostasis issues. This is correct because in my experience surgical wounds in this location on swine do not heal well due to locomotion issues.

Author's response: No adjustments from the comments provided by Reviewer 2 were required.

3) Veterinarian review

Were animals used humanely and was the appropriate anesthesia or analgesia provided for potentially painful procedures?

Yes, animals were used humanely and appropriate anesthesia was used for what appeared to be non-survival surgical procedures. However, this needs to be more clearly identified both in the video and the paper that was submitted that this was a non-survival surgical procedure.

Author's response: A statement was added to the video introduction indicating this is a non-recovery procedure. Additionally, while these are non-recovery procedures, statements indicating "surgical sterility" tissue preparation were added to the manuscript (Protocol sections 1.6 and 1.8).

Please provide additional comment, if necessary.

There was no mention of the potential for nerve damage for this approach which can be a significant complication associated with this access. This can be added to the text in the discussion section and does not need to be added to the video. It should be emphasized in the Tissue Dissection section under 2.1 that dissection of the nerve from the artery and vein needs to be specifically gentle/careful especially if this is done as a survival procedure.

Author's response: The information was added to the manuscript (lines 176-177, and 345).

I didn't have any other significant comments and the authors have done a very good job describing this technique and the viewers should find this video very helpful.

Author's response: The information requested in the table (see below) was added and edits completed

Please be specific in your comments. If possible, divide your comments into 2 categories:
a) Absolutely not acceptable - for serious errors and deviations from the animal research standards.
b) Improvement requires - for minor deviations, missing parts, etc....

For each comment, please specify if the changes in video are required, or if only changes in the complementary text are necessary. **Obviously, changes in the video are more difficult so it is important**

Example	2:20 – 2:34	Name of drug used for anesthesia is not mentioned	No	Yes	
1	1:00	There was no mention of the anesthesia that was used and whether this was a sterile or non-sterile procedure. It appears that this was a non-survival procedure and this should have been noted at the very beginning of the video	Yes	No	Indicate in the video what anesthesia was used and if this was a survival or non-survival surgical procedure.
2	5:00	Indicate what suture material was used to enhance catheter stability?	No	Yes	Add the suture type to the text

to note if changes in the text are sufficient. Please use the chart below to provide details on each issue (replace examples place holder

Click here to access/download

Video Produced by Author: Less than 50 MB

Place Holder for Video.docx