

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.

Author's response: *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.

Author's response: *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).

Author's response: *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.

Author's response: *The required video edits were completed.*

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

Author's response: *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.

Author's response: *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.

Author's response: *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.

Author's response: *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.

Author's response: 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.

Author's response: 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

Author's response: 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: $MAP = 1/3 \text{ systolic pressure} + 2/3 \text{ diastolic pressure}$ and is not an equal average between systolic and diastolic pressure- which seems as the most intuitive form for the calculation (Ref- laizzo 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 catheterization 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.

Author's response: *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.

Author's response: *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 too 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.

Author's response: *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.

Author's response: *Active video for set up and monitoring was not acquired during the procedure. We have however, provided a still image highlighting clinical parameters monitored through the procedure. A corresponding statement was added to the manuscript (protocol section 4.0; subsection 4.1, lines 239-242).*

Minor Concerns:

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

Author's response: *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