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Title: Structured Exercise Regimen in Pulmonary Hypertension-Right Ventricular Failure in an Ovine Model

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Author Questionnaire

- 1. Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done!**

- 3. Filming location:** Will the filming need to take place in multiple locations? **No**

- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **Yes**

Current Protocol Length

Number of Steps: 17

Number of Shots: 58

Interviews

Videographer: Obtain headshots for all authors available at the filming location.

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Blake Bulard, MD:** We are studying the effects of exercise on the pathophysiology of pulmonary hypertension-right ventricular failure in an ovine model.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Blake Bulard, MD:** Exercise effects in PH-RVF are poorly characterized due to limited tolerance and cardiopulmonary decompensation risk; this protocol enables controlled assessment.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Andrew Kumpfbeck, MD:** This protocol allows us to directly measure hemodynamic values in real time while exercising.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Andrew Kumpfbeck, MD:** A key challenge in this protocol is maintaining port access during exercise, particularly preserving consistent pressure in the PA cuff.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.5. **Andrew Kumpfbeck, MD:** One parameter we hope to measure in greater detail in the future is oxygen consumption during exercise and recovery.
 - 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

~~What problem in your field of research does your JoVE article address?~~

1.6 Blake Bulard, MD, Post Doctoral Research Fellow, Vanderbilt University Medical Center:
(authors will present their testimonial statements live)

1.6.1 INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~What is the key takeaway you hope viewers remember after watching your video article?~~

1.7 Andrew Kumpfbeck, MD, Post Doctoral Research Fellow, Vanderbilt University Medical Center:
(authors will present their testimonial statements live)

1.7.1 INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at Vanderbilt University Medical Center

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

2. Preparation of Heparinized Flushes and Pulmonary Artery (PA) Monitoring Setup

Demonstrator: Blake Bulard

2.1. To begin, collect the required reagents and set up the workspace for the experiment [1]. Take three normal saline flushes, remove 1 milliliter from each [2], and add 1 milliliter of heparin solution containing 1000 units to each syringe [3].

2.1.1. WIDE: Talent arranging supplies and preparing syringes in the workspace.

2.1.2. Talent picking up three normal saline flush syringes and removing 1 mL of saline from each syringe.

2.1.3. Talent adding 1 mL of heparin into each syringe.

2.2. Prepare two 10-milliliter syringes, one for waste and one for blood collection and five 1 milliliter syringes for blood gas collection [1]. Using a 5-milliliter syringe, draw up 5 milliliters of heparin solution containing 5000 units heparin for locking the right ventricular or RV (R-V) line [2]. Reconstitute 2 milligrams of alteplase with 2.2 milliliters of sterile water, for use if needed [3].

2.2.1. Talent labeling and placing two 10 milliliter syringes and five 1 milliliter syringes on the work bench.

2.2.2. Talent drawing heparin into a 5-mL syringe.

2.2.3. Talent adding 2.2 mL sterile water to 2mg alteplase.

2.3. Obtain one 1-milliliter syringe and one 5-milliliter syringe and fill both with hypertonic saline using a 20 to 22 gauge needle [1]. ~~Use the 1-milliliter syringe to inflate the cuff [2] and the 5-milliliter syringe to prime the line [3].~~

Author's/ Videographer's NOTE: These steps will be done later.

2.3.1. Talent filling the one 1-mL syringe and one 5-mL syringe with hypertonic saline.

~~2.3.2. Talent inflating the cuff using the 1 mL syringe~~

Authors's NOTE: This step is actually done later on step 4.6.3 and therefore doesn't need to be done here.

~~2.3.3. Talent priming the line using the 5 mL syringe~~

Authors's NOTE: This step is actually done later on step 2.4.4 and therefore doesn't need to be done here.

- 2.4. Obtain a pressure transducer and connect the female Luer end of pressure tubing to the male Luer end of the transducer [1]. Confirm that the bottom Luer end of the transducer is capped [2]. Connect the tubing to a three-way stopcock and attach a 22-gauge Huber needle [3]. Flush the line with hypertonic saline until fluid exits the needle [4]. Adjust the stopcock to lock toward the needle and then turn the three-way stopcock ~~towards the~~ away from the blue cap to open access to the cuff [5].

Authors's NOTE: In [5], please change "towards the" to "away from".

- 2.4.1. Talent connecting tubing to a pressure transducer.
- 2.4.2. Talent checking whether the bottom port of the transducer is capped.
- 2.4.3. Talent attaching tubing and Huber needle via stopcock.
- 2.4.4. Talent flushing saline through the line until it exits the needle.
- 2.4.5. Talent adjusting the stopcock towards the needle and then rotating the three-way stopcock ~~towards the~~ away from the blue cap.

3. Heparinized System Setup and Animal Preparation

Demonstrators: Andrew Kumpfbeck, Blake Bulard, Bilaal Said, Benjamin Schanz, Sriya Bapatla

- 3.1. Remove 2 milliliters of normal saline from a 1 liter bag and add 2 milliliters of heparin solution containing 2000 units heparin [1]. Place the saline bag into a pressure bag insert it into an access bag with an intravenous spike [2]. Hang the pressure bag about two meters above the ground and turn the three-way stopcock on the pressure bag so the handle is perpendicular to the tubing [3]. Inflate the bag using the hand pump until the pressure indicator turns green at approximately 250 millimeters of mercury [4].

Authors's NOTE: In [3], Please add, "Hang the pressure bag about two meters above the ground and".

- 3.1.1. Talent withdrawing 2 milliliters of normal saline from a 1 L bag and injecting 2 mL of heparin into the same bag.
 - 3.1.2. Talent placing the saline bag into a pressure bag and then an access bag with an intravenous spike.
 - 3.1.3. Talent **hanging the bag and** adjusting the three-way stopcock to a perpendicular position.
 - 3.1.4. Talent inflating the pressure bag using the hand pump until the indicator shows green.
- 3.2. ~~Turn the three-way stopcock towards the bag to maintain pressure and place the bag on the intravenous pole approximately 2 meters above the ground [1]. Attach each tube to its designated location [2].~~ Connect the male end of the intravenous line to the

female end of the transducer [3]. Connect the female Luer end of the pressure tubing to the male end of the transducer [4]. Connect the male end of the tubing to the female Luer end of the stopcock [5].

Author's NOTE: This action was already performed in 3.1.1

Author's NOTE: All of the steps in 3.2.2 (TEXT ON A PLAIN BACKGROUND) were performed and should be in the video as they are key to proper set up.

NOTE: VO has been added for the additional shots.

3.2.1. Talent turning the stopcock toward the bag and hanging the pressure bag on an intravenous pole at the specified height.

~~3.2.2. TEXT ON A PLAIN BACKGROUND:~~

~~The tubing and designated location:~~

~~1. Connect the intravenous line (male end) to the transducer (female end)~~

~~2. Connect the pressure tubing (female Luer end) to the transducer (male end)~~

~~3. Connect the tubing (male end) to the stopcock (female Luer end)~~

Added shot 3.2.3: Talent connecting the male end of the intravenous line to the female end of the transducer.

Added shot 3.2.4: Talent connecting the female Luer end of the pressure tubing to the male end of the transducer.

Added shot 3.2.5: Talent connecting the male end of the tubing to the female Luer end of the stopcock.

3.3. Connect a 22-gauge Huber needle to the male end of the three-way stopcock [1]. Open the heparin bag intravenous line to flush the entire line until heparinized saline exits the Huber needle [2]. Turn the three-way stopcock toward the Huber needle to lock it [3].

3.3.1. Talent attaching the 22-gauge Huber needle to the stopcock.

3.3.2. Talent opening the intravenous line from the heparinized saline bag to flush the line until fluid emerges from the Huber needle.

3.3.3. Talent turning the stopcock toward the Huber needle to lock the line.

3.4. Transport a sheep model of pulmonary hypertension-RV failure instrumented with a pulmonary artery or PA (P-A) cuff and RV pressure port, ready for exercise testing, to the exercise room [1]. ~~Use a transport cage to transfer the sheep to the exercise room [2].~~ Place a halter and lead the sheep onto the treadmill [3]. Use the halter to position the sheep at the front of the treadmill with front legs on a small crate [4].

Author's NOTE: Change to "place the halter and" lead

Author's/ Videographer's NOTE: Please omit 3.4.1 and move up steps accordingly. There will only be three steps in this section.

NOTE: VO merged as per Author's feedback.

~~3.4.1. WIDE: Shot of the sheep ready for exercise testing~~

- 3.4.2. Talent moving the sheep to the exercise room in a transport cage.
- 3.4.3. Talent placing a halter and guiding the sheep onto a treadmill and placing a halter.
- 3.4.4. Talent positioning the sheep with front legs on a crate.

- 3.5. Palpate the ports and clean the skin with alcohol wipes [1]. Use a Huber needle to access the PA port [2]. ~~Repeat the same procedure to access the RV port [3].~~

Author's NOTE: The sheep was agitated during this step (3.5.3) and our veterinarian onsite ask that this step be omitted. This is not an issue since accessing the PA and RV port is the same procedure.

Videographer's NOTE: Shot 3-5-3 – There is a concern that the animal seemed to be in distress during the needle insert - suggest using a freeze frame here

- 3.5.1. Talent palpating and cleaning the port site.
- 3.5.2. Talent inserting a Huber needle into the PA port.
- ~~3.5.3. Shot of the talent inserting a Huber needle into the RV port.~~

- 3.6. After opening **LabChart** home page, select **New**, click on **Set up**, select **Devices and Channels**, set the number of channels to 4 and rename channel 1 to 4 as PA cuff pressure, RV pressure, RV systolic pressure, and heart rate [1]. Select **Cyclic measurements**, go to Source and select Channel 2 RV pressure, measurement to **Maximum** and click OK [2]. Repeat process for Channel 4 Heart Rate but change measurement to **Rate** [3]. For channel 1 and 2, confirm that **No calculation** is selected [4].

- 3.6.1. SCREEN: 70644_ 11-25-59.mp4 00:19-00:56 *Video Editor: Please speed up the footage to match the VO.*
- 3.6.2. SCREEN: 70644_ 11-25-59.mp4 00:58-01:07
- 3.6.3. SCREEN: 70644_ 11-25-59.mp4 01:08-01:18
- 3.6.4. SCREEN: 70644_ 11-25-59.mp4 01:19-01:23

- 3.7. Ensure correct access orientation with the PA port positioned closer to the head and the RV port positioned closer to the tail [1]. **Position transducers between the sheep and computer at the heart level and connect both pressure transducers to the interface cable** [2].

Authors' NOTE: 3.7.1 was not filmed. Please show Figure 3B from the manuscript.

- 3.7.1. LAB MEDIA: Figure 3B. ~~Talent adjusting and verifying the placement of PA and RV ports on the sheep.~~
- 3.7.2. Talent positioning the transducers between the computer and the sheep at heart level and connecting both pressure transducers to the interface cable.

4. Hemodynamic Monitoring and Blood Sampling During Exercise

Demonstrator: Blake Bulard, Andrew Kumpfbeck, Bilaal Said, Benjamin Schanz, Sriya Bapatla

- 4.1. Once the transducers are zeroed and calibrated, click **Start** to begin recording the RV and PA cuff pressure waveforms at 400 hertz [1]. Turn the stopcock upward to transduce the PA cuff pressure waveform [2]. Then, turn the stopcock off toward the sheep to close the waveform [3]. ~~Place the cap on the three-way stopcock to prevent backflow [4].~~

Author's NOTE: Please omit 4.1.4.

4.1.1. SCREEN: 70644 11-45-21 00:03-00:06 *Video editor: Please highlight the start button at the top right corner*

4.1.2. Talent turning the stopcock upward to open the PA cuff line. *Video editor: Please add Figure 4A as an inset*

4.1.3. Talent turning the stopcock toward the sheep to close the waveform. *Video editor: Please add Figure 4B as an inset*

~~4.1.4. Talent placing caps on the transducer ends.~~

- 4.2. Place one heparin flush on the three-way stopcock for the RV line and flush the entire line into the RV port [1]. Gently draw back on the syringe until dark blood returns [2].

4.2.1. Talent attaching the heparin flush to the three-way stopcock and flushing the line into the RV port.

4.2.2. Talent pulling back on the syringe and observing dark blood return.

- 4.3. ~~Power flush with an additional 5 to 10 milliliters of heparinized saline if blood is difficult to draw [1]. Then, use a large empty syringe such as 20 milliliters or 30 milliliters to draw back the blood [2]. If clogging persists, inject 1 to 2 milligrams of alteplase into the line and wait approximately 10 minutes [3]. Open the three-way stopcock connected to the RV line to transduce RV pressure and waveform and observe waveform variation at rest, exercise, and recovery speeds [4].~~

Author's NOTE: 4.3 was omitted as we don't need to show clogging and this is more of a note in the procedural outline. However, can pan to showing LAB MEDIA 4C as an inset as this will show what a correct RV waveform looks like.

NOTE: LABMEDIA is not included because no corresponding action has been captured.

~~4.3.1. Talent forcefully flushing the line with heparinized saline.~~

~~4.3.2. Talent drawing back using a large syringe.~~

~~4.3.3. Talent injecting alteplase into the line.~~

~~4.3.4. Talent turning the stopcock to open the RV line for pressure transduction. *Video editor: Please add LAB MEDIA Figure 4C as an inset.*~~

- 4.4. Turn the stopcock off toward the transducer [1]. First use an empty 10-milliliter syringe to draw 10 milliliters of RV blood [2]. Then, use a 1-milliliter syringe to draw 0.2 to 0.4 milliliters of RV blood as the sample [3]. ~~Return the 10 milliliters of RV blood back to the sheep [4].~~

Videographer's NOTE: Shot 4-4-1 has 2 takes.

Videographer's NOTE: Shot 4-4-4 has been removed.

- 4.4.1. Talent turning the stopcock toward the transducer.
- 4.4.2. Talent withdrawing 10 mL of RV blood using a 10-mL syringe.
- 4.4.3. Talent collecting 0.2 to 0.4 mL of blood using a 1-mL syringe as the sample for blood gas analysis.
- 4.4.4. ~~Talent reinfusing the 10 mL of blood into the RV line.~~
- 4.5. Turn the stopcock to the upward position [1]. Pull the ~~white~~ blue tab on the RV pressure transducer to open the valve until all blood clears from the pressure line [2]. Start the first exercise speed by setting the treadmill knob to 30, corresponding to 0.46 meters per second, and maintain for 10 minutes [3]. Open the PA cuff line periodically during the 10-minute interval to transduce PA cuff pressures [4].

NOTE: Color changed from white to blue based on author's input.

- 4.5.1. Talent turning the stopcock upward.
- 4.5.2. Talent pulling the ~~white~~ blue tab and flushing the line until clear.
- 4.5.3. Talent adjusting the treadmill knob to 30 to initiate exercise.
- 4.5.4. Shot of the talent opening the PA cuff stopcock during exercise.
- 4.6. Near the end of the 10-minute interval, obtain blood gas as demonstrated previously [1]. For treadmill settings of 40, 50, then 30, and finally 0, repeat the blood collection, flushing, and exercise procedure [2]. Use 1 milliliter of hypertonic saline to inflate the cuff to 150 to 200 millimeters of mercury [3]. Transduce RV pressure for a few minutes after inflation to ensure tolerance [4].
- 4.6.1. Shot of the talent collecting a blood sample near the end of the exercise interval.
- 4.6.2. Talent adjusting treadmill settings sequentially through the specified speeds.
- 4.6.3. Talent injecting hypertonic saline to inflate the cuff.
- 4.6.4. The talent monitoring the RV pressure waveform in **LabChart** after cuff inflation.
- Videographer: Please zoom in to show the monitor*

Results

5. Results

5.1. Mixed venous oxygen saturation remained relatively stable at maximum treadmill speed from week 1 to week 8 [1]

5.1.1. LAB MEDIA: Figure 5A. *Video editor: Highlight the red maximum speed line at week 1 and week 8 to show that the values stayed at a similar level.*

5.2. RV systolic pressure increased from week 1 to week 8 [1].

5.2.1. LAB MEDIA: Figure 5B. *Video editor: Highlight the red maximum speed line at week 1 and week 8, and emphasize the higher point at week 8.*

5.3. The distance traveled during exercise increased from week 1 to week 8 [1].

5.3.1. LAB MEDIA: Figure 5C. *Video editor: Highlight the bar at week 1 and the bar at week 8 and emphasize that the week 8 bar is taller.*

5.4. PA cuff pressure at week 8 increased to 777 ± 98 millimeters of mercury [1].

5.4.1. LAB MEDIA: Figure 5D. *Video editor: Highlight the bar at week 8 and its error bar. Please add a label '777 ± 98' to mark this bar.*

5.5. At the selected treadmill speeds, mixed venous oxygen saturation decreased [1] and RV systolic pressure increased as exercise speed increased [2].

5.5.1. LAB MEDIA: Figure 6. *Video editor: Highlight dip in the blue line labelled "SvO2" corresponding to the speeds 0.46, 0.78 and 1.1 meters per second*

5.5.2. LAB MEDIA: Figure 6. *Video editor: Highlight the three points in the red line labelled "RVSP" corresponding to the speeds 0.46, 0.78 and 1.1 meters per second*