FINAL SCRIPT: APPROVED FOR FILMING

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Title: Evaluation of Right Ventricular Function in Experimental Models of Pulmonary Arterial Hypertension

Authors and Affiliations:
Renata Trabach Santos, Maria Eduarda de Sá Freire Onofre, Victória Marques Barbosa, Nazareth Novaes Rocha, Patricia Rieken Macedo Rocco, Fernanda Ferreira Cruz, Pedro Leme Silva

Laboratory of Pulmonary Investigation, Institute of Biophysics Carlos Chagas Filho, Universidade Federal do Rio de Janeiro

Corresponding Authors:
Pedro Leme Silva pedroleme@biof.ufrj.br
Email Addresses for All Authors:
Renata Trabach Santos rtrabach@biof.ufrj.br
Maria Eduarda de Sá Freire Onofre meduarda.onofre@biof.ufrj.br
Victória Marques Barbosa vick.marques13@biof.ufrj.br
Nazareth Novaes Rocha nn_rocha@hotmail.com
Patricia Rieken Macedo Rocco prmrocco@biof.ufrj.br
Fernanda Ferreira Cruz ffcruz@biof.ufrj.br
Pedro Leme Silva pedroleme@biof.ufrj.br

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? **Yes, in 2 different rooms of the same building (30 m apart)**

Current Protocol Length
Number of Steps: 27
Number of Shots: 55

Introduction

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

1.1. **Renata Trabach Santos:** We explore regenerative therapies—particularly mesenchymal stromal cells, extracellular vesicles, and mitochondria—to reverse vascular remodeling and improve cardiopulmonary function in experimental pulmonary arterial hypertension.

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

What are the current experimental challenges?

1.2. **Maria Eduarda de Sá Freire Onofre:** Invasive procedures like right ventricular puncture and heart dissection are terminal, limiting longitudinal studies. Additionally, variability in echocardiographic measurements can affect reproducibility across different operators and time points.

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

What advantage does your protocol offer compared to other techniques?

1.3. **Maria Eduarda de Sá Freire Onofre:** Our protocol combines non-invasive echocardiography with invasive hemodynamic measurements and post-mortem analysis, providing comprehensive assessment of pulmonary hypertension progression and right ventricular remodeling in rats. This integrated approach enhances data reliability and reduces the number of animals needed.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.10*

What research questions will your laboratory focus on in the future?

1.4. **Victoria Marques Barbosa:** In the future, we aim to better explore brain-heart-lung interaction and cognitive deficits in humans with pulmonary arterial hypertension, to understand clinical relevance and potential underlying mechanisms.

1.4.1. 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):

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.5. **Renata Trabach Santos:** Publishing with JoVE standardizes right ventricular data collection in pulmonary hypertension models, enhancing reproducibility, clarifying protocols through visualization, and supporting consistent preclinical research for translational studies.

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

Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE? (This could include increased collaborations, citations, funding opportunities, streamlined lab procedures, reduced training time, cost savings in the lab, or improved lab productivity.)

1.6. **Pedro Leme Silva:** After publishing JoVE about standardization of right ventricular functional and hypertrophy data, we expect to increase international collaborations while reducing training time and improve lab productivity. This is a unique opportunity to show "how to" without scientific borders.

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

Videographer: Please capture the testimonials in both Portuguese and English

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee of the Health Sciences Center, Federal University of Rio de Janeiro

Protocol

2. Echocardiographic and Analysis of Cardiac Function in Mice Models

Demonstrators: Renata Trabach Santos, Nazareth Novaes Rocha, Maria Eduarda de Sá Freire Onofre, Victória Marques Barbosa

2.1. To begin, use gauze dressing soaked in detergent and water to wet the fur on the chest of the anesthetized animal **[1-TXT]**. With a razor blade attached to Kelly forceps, shave the fur from the animal's chest **[2]**. Apply a generous amount of conductive gel to the animal's chest **[3]**.

2.1.1. WIDE: Talent wetting the fur on the animal's chest using gauze dressing soaked in detergent and water. **TXT: Anesthesia: 2.5% isoflurane inhalation**

2.1.2. Talent shaving the fur from the animal's chest using a razor blade attached to Kelly forceps.

2.1.3. Talent applying conductive gel over the shaved chest area.

2.2. Press the **2D** button on the **control panel [1]**. Place the transducer between the third and fifth intercostal space to acquire a short-axis view **[2]**.

2.2.1. SCREEN: Pressing the 2D button on the control panel interface.
NOTE: Filmed by videographer

2.2.2. Talent placing the transducer between the third and fifth intercostal space.

2.3. Then direct the transducer toward the animal's left shoulder at a 90-degree angle relative to the sternum to capture a cross-sectional image of the base of the heart, showing the right ventricle, aortic valve, pulmonary valve, and left atrium **[1]**.

2.3.1. SCREEN: Short-axis cross-sectional image showing the base of the heart with relevant anatomical structures.
NOTE: Filmed by videographer

2.4. Press the **Freeze** button on the **control panel** to freeze the image **[1]**. Then press the **Save** button on the control panel to save the image **[2]**.
AUTHOR'S NOTE: Shots 2.4.1-2.4.2 filmed by videographer

2.4.1. SCREEN: Pressing Freeze button on the control panel to freeze the captured image.

2.4.2. SCREEN: Pressing Save button to store the frozen image.

2.5. When the correct angle is located, hold that position and press the **PW (Pulse Wave)** *(P-W-Pulse Wave)* button to activate pulsed-wave spectral Doppler mode **[1]**. Use the **trackball** on the control panel to move the yellow cursor in any direction on the screen and place it over the pulmonary valve **[2]**.
AUTHOR'S NOTE: Shots 2.5.1-2.5.2 filmed by videographer

2.5.1. SCREEN: Pressing PW button to initiate spectral Doppler mode.
2.5.2. SCREEN: Using trackball to position the yellow cursor accurately over the pulmonary valve on-screen.

2.6. Press the **Five-button** on the **control panel** to acquire the blood flow curve across the pulmonary valve **[1]**. Now, position the transducer between the third and fifth intercostal space to acquire a long-axis view **[2]**.
2.6.1. SCREEN: Blood flow curve being displayed after pressing 5 button.
NOTE: Filmed by videographer
2.6.2. Talent adjusting the transducer for long-axis positioning.

2.7. Direct the transducer toward the animal's right shoulder at a 45 to 60-degree angle relative to the sternum, capturing a longitudinal section showing the left ventricle, right ventricle, left atrium, mitral valve, and aortic valve **[1]**.
2.7.1. SCREEN: Longitudinal heart section displayed with labeled anatomical features.
NOTE: Filmed by videographer

2.8. Press the **Freeze** button on the control panel to freeze the image **[1]**. Then press the **Save** button on the control panel to store the image **[2]**.
AUTHOR'S NOTE: Shots 2.8.1-2.8.2 filmed by videographer
2.8.1. SCREEN: Freezing the long-axis image.
2.8.2. SCREEN: Saving the frozen image on the system.

2.9. Press the **END EXAM** button on the control panel to conclude the imaging session and store the images in the patient database **[1]**.
2.9.1. SCREEN: Pressing END EXAM to finish the experiment and confirm saving.

2.10. For electrocardiography, select **SEARCH** *(search)* on the screen and choose the appropriate **animal ID** *(I-D)* from the database to begin analysis **[1]**. Press the **SONOVIEW** *(sono-view)* button on the control panel **[2]**. Click once inside the image area on-screen to start the analysis **[3]**.
AUTHOR'S NOTE: Shots 2.10.1-2.10.3 filmed by videographer
2.10.1. SCREEN: Selecting SEARCH and choosing correct animal ID from the database.
2.10.2. SCREEN: Pressing SONOVIEW on the control panel.
2.10.3. SCREEN: Clicking once within the image window to initiate analysis.

2.11. Use the trackball to select the short-axis image in pulsed-wave spectral Doppler mode **[1]**.
2.11.1. SCREEN: Navigating with the trackball to select short-axis image in Doppler mode.
NOTE: Filmed by videographer

2.12. Now press the **Calculator** button and select **Heart Rate (HR)** *(Heart-Rate-H-R)* on the screen **[1]**. Use the trackball to draw cursor lines peak to peak of two curves. Repeat this three times to obtain the average **[2]**.

NOTE: Filmed by videographer

2.12.1. SCREEN: Selecting HR under Calculator menu.

2.12.2. SCREEN: Placing cursor from peak to peak three times to calculate average HR.

2.13. Press the **Calculator** button again and choose **Pulmonary Valve (PV)** *(Pulmonary-Valve -P-V)* and then **PV Acceleration Time / Ejection Time (PV AccT/ET)** *(P-V-Acceleration Time-Ejection-Time)* on the screen **[1]**. Use the trackball to place a cursor from the start to the peak, and then from the start to the end of the same curve. Repeat three times to obtain the average **[2]**.

AUTHOR'S NOTE: Shots 2.13.1-2.13.2 filmed by videographer

2.13.1. SCREEN: Selecting PV and PV AccT/ET from Calculator menu.

2.13.2. SCREEN: Drawing lines from start to peak and start to end of the curve to determine acceleration time and ejection time.

2.14. Press the **Save** button on the control panel to save the results **[1]**. Using the trackball, select the long-axis image from the upper right section of the screen **[2]**.

NOTE: Filmed by videographer

2.14.1. SCREEN: Saving all computed parameters using Save.

2.14.2. SCREEN: Navigating with the trackball to select the long-axis image for measurement.

2.15. Press the **Calculator** button on the control panel and select the **Right Ventricular Outflow Tract (RVOT)** *(Right-Ventricular-Outflow-Tract-R-V-O-T)* parameter and then the **RVOT Diameter (RVOT DIAM)** *(R-V-O-T-Diameter)* on the screen **[1]**. Using the trackball, position the cursor from one wall of the right ventricle to the opposite wall. Perform this three times to obtain the average **[2]**.

AUTHOR'S NOTE: Shots 2.15.1-2.15.2 filmed by videographer

2.15.1. SCREEN: Selecting RVOT and RVOT DIAM under Calculator menu.

2.15.2. SCREEN: Placing cursor from one wall of the right ventricle to the other to measure diameter.

2.16. For right ventricular puncture, first tracheostomize an anesthetized animal **[1]**. Ensure the computer is powered on, the LabView software is open, and the baseline is configured for signal acquisition **[2]**.

2.16.1. Shot of a tracheostomized animal.

2.16.2. SCREEN: 2.16.2.mp4. 00:03-00:26, 00:42-00:47

2.17. After locating the right ventricle ~~using the interventricular branch of the coronary arteries as a reference [1].~~ In the software, verify that signal acquisition has begun. Click the **Save** button on the screen and enter the animal's ID **[1]**.

2.17.1. ~~SCOPE/SCREEN: The interventricular coronary artery branch and the right ventricle are being seen~~.
NOTE: Shot not provided
2.17.2. SCREEN: 2.17.2-and-2.17.3.mp4 00:00-00:22

2.18. Use a heparinized saline-filled 19-gauge scalp vein set to puncture slightly above the anatomical reference point, taking care not to insert the needle too deeply **[1]**. Verify accuracy of the puncture by observing the pressure values **[2]**.
2.18.1. Talent puncturing with 19-gauge scalp vein set at precise anatomical location.
2.18.2. Shot of the pressure values.
AND
TEXT ON PLAIN BACKGROUND:
RVSP in control animals: 10–20 mmHg
RVSP in animals with pulmonary arterial hypertension: up to 60 mmHg

Acquired pressure >80 mmHg indicates that the cannula probably reached the left ventricle

2.19. Record at least 10 stable pressure wave peaks to ensure data reliability **[1]**. At the end of signal acquisition, administer heparin using a heparinized syringe for blood collection via puncture of the left ventricle or abdominal vena cava **[2]**.
2.19.1. SCREEN: 2.19.1.mp4 00:03-00:20
2.19.2. Talent puncturing and drawing blood from the left ventricle or abdominal vena cava using a heparinized syringe.

2.20. For data analysis, open the MATLAB *(mat-lab)* program, click the **Browse for Paste** button and select the file on the computer's internal hard drive that contains the correct code **[1]**. On the left side of the MATLAB screen, observe the list of files that include codes for each channel recognized by the transducer **[2]**.
2.20.1. SCREEN: 2.20.1-up-to-2.24.1.mp4 00:00-00:18
2.20.2. SCREEN: 2.20.1-up-to-2.24.1.mp4 00:18-00:20
Video Editor: Please freeze frame here

2.21. Choose the code corresponding to the selected channel configuration used earlier in the experiment **[1]**. Press the **Run** button and choose the **.bin** *(dot-bin)* file on the external hard drive for analysis **[2]**.
2.21.1. SCREEN: 2.20.1-up-to-2.24.1.mp4 00:21-00:23
Video Editor: Please freeze frame here
2.21.2. SCREEN: 2.20.1-up-to-2.24.1.mp4 00:23-00:31

2.22. Select the most uniform section of the curve by clicking once at the beginning and once at the end, identifying the portion with visually equal peaks and troughs **[1]**.

Then select a 10-second segment of the curve by clicking once at 0 seconds and once at 10 seconds **[2]**.

2.22.1. SCREEN: 2.20.1-up-to-2.24.1.mp4 00:32-00:39

2.22.2. SCREEN: 2.20.1-up-to-2.24.1.mp4 00:40-00:46

2.23. Adjust the baseline by subtracting the lowest trough value in the command window and pressing **ENTER** on the keyboard. The software will automatically generate all relevant data **[1]**. Observe the data in two columns that are time of peak and pressure value **[2]**.

2.23.1. SCREEN: 2.20.1-up-to-2.24.1.mp4 00:47-01:14

2.23.2. SCREEN: 2.20.1-up-to-2.24.1.mp4 01:18-01:23

2.24. Copy and paste the systolic pressure values into a **.txt** *(dot-T-X-T)* file and remove the time values **[1]**. Replace all periods with commas in the file to enable calculation of the average **[2]**. Select 10 peak pressure values and calculate their average **[3]**.

2.24.1. SCREEN: 2.20.1-up-to-2.24.1.mp4 01:25-01:37, 01:42-01:54

2.24.2. SCREEN: 2.24.2-and-2.24.3.mp4 00:17-00:25

2.24.3. SCREEN: 2.24.2-and-2.24.3.mp4 00:07-00:12,00:35-00:44

2.25. For right ventricular hypertrophy index,measure and record the animal's body weight at the start of the experiment **[1]**. Measure the right ventricular systolic pressure, then euthanize and remove the thoracic organs **[2]**.

2.25.1. Talent weighing the animal and documenting the value.

2.25.2. Shot of the extracted organs

2.26. Dissect the aorta, pulmonary artery, vena cava, and pulmonary veins from the base of the heart **[1]**. Then dry the entire heart using gauze **[2]** and record the dry weight using a precision balance **[3]**.

2.26.1. Talent isolating and cutting the major vessels from the heart base.

2.26.2. Talent drying the whole heart.

2.26.3. Shot of the dried heart being placed on a precision balance.

2.27. After dissecting and removing the atriums. separate the right ventricle from the left ventricle along with the septum **[1]**. Record the weights of the dried right ventricle and the left ventricle plus septum separately using the precision balance **[2]**.

2.27.1. Talent dissecting and isolating the right and left ventricles with septum.

2.27.2. Talent drying and weighing each cardiac component individually.

Results

3. Results

3.1. The PAT *(Pat)* to PET *(Pet)* ratio was significantly reduced in pulmonary arterial hypertension animals compared to controls, indicating increased pulmonary vascular resistance and altered blood flow pattern in the pulmonary artery **[1]**.

3.1.1. LAB MEDIA: Figure 5B. Video editor: Highlight the bar for PAH.

3.2. The right ventricular outflow diameter was increased in pulmonary arterial hypertension animals compared to controls, suggesting right ventricular hypertrophy as an adaptation to elevated pulmonary pressure **[1]**. Representative echocardiography images showed visibly enlarged right ventricular outflow diameters and altered flow patterns in pulmonary arterial hypertension animals relative to controls **[2]**.

3.2.1. LAB MEDIA: Figure 5C. *Video editor: Highlight the taller bar for PAH*.

3.2.2. LAB MEDIA: Figure 5A. *Video editor: Highlight the images in the PAH panel*

3.3. Right ventricular systolic pressure was significantly elevated in pulmonary arterial hypertension animals compared to controls, reflecting the increased pulmonary vascular resistance **[1]**. The pressure waveform recordings showed higher peaks in pulmonary arterial hypertension animals, consistent with elevated RVSP **[2]**.

3.3.1. LAB MEDIA: Figure 6B. *Video editor: Highlight the bar for PAH*

3.3.2. LAB MEDIA: Figure 6A. *Video editor: Highlight the graph labeled PAH*

3.4. Right ventricular hypertrophy index was significantly elevated in pulmonary arterial hypertension animals compared to controls **[1]**.

3.4.1. LAB MEDIA: Figure 7. *Video editor: Highlight the bar for PAH.*

Pronunciation Guide:

1. **Pulmonary**

Pronunciation link:
https://www.merriam-webster.com/dictionary/pulmonary
IPA: /ˈpʊl.məˌnɛr.i/
Phonetic Spelling: puhl-muh-nair-ee

2. Echocardiography

Pronunciation link:
https://www.merriam-webster.com/dictionary/echocardiography
IPA: /ˌɛk.oʊˌkɑːr.diˈɑː.grə.fi/
Phonetic Spelling: eh-koh-kar-dee-ah-gruh-fee

3. Mesenchymal

Pronunciation link:
https://www.merriam-webster.com/dictionary/mesenchymal
IPA: /ˌmɛˈzɛŋ.kɪ.məl/
Phonetic Spelling: meh-zen-ki-muhl

4. Heparin

Pronunciation link:
https://www.merriam-webster.com/dictionary/heparin
IPA: /ˈhɛp.ə.rɪn/
Phonetic Spelling: hep-uh-rin

5. Tracheostomize

Pronunciation link:
No confirmed link found
IPA: /ˌtreɪ.kiˈɑː.stəˌmaɪz/
Phonetic Spelling: tray-kee-ah-stuh-myz

6. Systolic

Pronunciation link:
https://www.merriam-webster.com/dictionary/systolic
IPA: /sɪˈstɑː.lɪk/
Phonetic Spelling: sih-staa-lik

7. Hypertrophy

Pronunciation link:
https://www.merriam-webster.com/dictionary/hypertrophy
IPA: /haɪˈpɝː.trə.fi/
Phonetic Spelling: hy-per-truh-fee

8. Hemodynamic

Pronunciation link:
https://www.merriam-webster.com/dictionary/hemodynamic
IPA: /ˌhiː.moʊ.daɪˈnæ.mɪk/
Phonetic Spelling: hee-moh-dai-na-mik

9. Intercostal

Pronunciation link:
https://www.merriam-webster.com/dictionary/intercostal
IPA: /ˌɪn.tɚˈkɑː.stəl/
Phonetic Spelling: in-ter-kaw-stuhl

10. Spectral Doppler

Pronunciation link:
No confirmed link found for the phrase as a whole
Spectral
https://www.merriam-webster.com/dictionary/spectral
IPA: /ˈspɛk.trəl/
Phonetic Spelling: spek-truhl

Doppler
https://www.merriam-webster.com/dictionary/Doppler
IPA: /ˈdɑː.plɚ/
Phonetic Spelling: dop-lur