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Title: Induction and Phenotyping of Acute Right Heart Failure in a Large Animal Model of Chronic Thromboembolic Pulmonary Hypertension

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Author Questionnaire:

1. Microscopy: Does your protocol involve video microscopy? N

2. Does your protocol include software usage? Y

If yes, we will need you to record using screen recording software to capture the steps. If you use a Mac, QuickTime X also has the ability to record the steps.

Authors: please upload all screen captured files to your project page.

Videographer: Please film the screen for all SCREEN shots as backup

3. Will the filming need to take place in multiple locations? **N**

Section - Introduction

Videographer: Interviewee Headshots are <u>required</u>. Take a headshot for each interviewee.

1. **REQUIRED Interview Statements (Said by you on camera): All interview statements may be edited for length and clarity.**

1.1. **<u>David Boulate</u>**: This model of acute right heart failure in the context of chronic thromboembolic pulmonary hypertension can help to better understand the pathophysiology of this clinically relevant situation.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (last one)

1.2. **<u>David Boulate</u>**: Induction of acute right heart failure by the mean of volume then pressure overload is easily reproducible and reproduces the main pathophysiological aspects of the corresponding clinical condition.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (last one)

Ethics title card: (for human subjects or animal work, does not count toward word length total)

1.3. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at Hospital Marie Lannelongue, Le Plessis-Robinson, France.

Section - Protocol

Video Editor: Please use the videographer's footage for the screen captures

2. Catheter Placement

2.1. After confirming an appropriate level of sedation **[1-TXT]**, perform a division of the right femoral vessels to introduce a fluid filled catheter **[2]** into the right femoral artery for continuous systemic pressure monitoring **[3]**.

2.1.1. WIDE: Talent confirming sedation **TEXT: See text for anesthesia/full pig preparation details**

2.1.2. CU: Jugular catheter insert

2.1.2B Added shot: extra check with fluoroscope

2.1.3. CU: Catheter being inserted Videographer NOTE: Use take 2, end of the purge

2.2. Next, make a 4-centimeter transverse incision at the groin **[1]** and insert a Beckman retractor into the incision **[2]**.

2.2.1. CU: Incision being made

2.2.2. CU: Retractor being inserted This shot is combined with 2.2.1

2.3. Using Debackey forceps and Metzenbaum scissors, divide the anterior face of the femoral vein and artery **[1]** and place a 20-gauge catheter into the femoral artery **[2]**.

2.3.1. CU: Vein and artery being divided Videographer NOTE: take 2 no slate, in the file name

2.3.2. CU: Catheter being placed Videographer NOTE: take 2, catheter in place

2.4. Then connect the catheter to a disposable transducer with a fluid filled catheter to obtain continuous systemic blood pressure monitoring **[1-TXT]**.

2.4.1. MED: Talent connecting catheter to transducer **TEXT: Maintain mean blood pressure at 60 mmHg**

2.5. Using an 18-gauge catheter and a fluoroscope with a C-arm and an anteroposterior view **[1]**, insert a guidewire into the femoral vein through the inferior vena cava **[2]**.

2.5.1. MED: Talent moving fluoroscope over/around pig Videographer NOTE: slated at the end
2.5.2. SCREEN: Guidewire being inserted into femoral vein *Videographer: Please film the screen*
Video Editor: Please use the videographer's footage for the screen captures

2.6. Place a balloon dilation catheter over the guidewire at the intrapericardial level **[1]** and place the visible markers of the balloon immediately above the diaphragm level **[2]**.

2.6.1. SCREEN: Catheter being placed over guidewire *Videographer: Please film the screen* Videographer NOTE: take 1: catheter insertion, take 2: screen
2.6.2. SCREEN: Marker(s) being placed above diaphragm *Videographer: Please film the screen*

2.7. Then remove the guidewire **[1]**.

2.7.1. SCREEN: Shot of catheter in place, then guidewire being removed *Videographer: Please film the screen*

3. Echocardiography

3.1. Immediately after the catheters have been placed, acquire an apical 5-chamber view under the xiphoid process in 2-dimensions **[1-TXT]** and in the tissue Doppler mode **[2]**. *Videographer: This step is important!*

3.1.1. WIDE: Talent obtaining apical 5-chamber view *Videographer: More Talent than pig in shot* **TEXT: Acquire each echocardiographic view in cine loop form for ≥ 3 cardiac cycles during end-expiratory apnea**
3.1.2. SCREEN: Apical 5-chamber view being acquired in tissue Doppler mode *Videographer: Please film the screen* Use 3.3.2 take 1

3.2. Acquire the parasternal short and long axis views on the right side of the sternum in 2D and tissue Doppler modes **[1]** and an image of the valvular flow using continuous and pulsed Doppler modes **[2]**.

3.2.1. SCREEN: 2D or Doppler scan being acquired *Videographer: Please film the screen*

3.2.2. SCREEN: Valvular flow image being acquired by continuous or pulsed Doppler *Videographer: Please film the screen*

3.3. Then acquire tissue Doppler signals of the lateral tricuspid annulus **[1]** and the lateral and septal mitral annulus **[2]**.

3.3.1. SCREEN: Lateral tricuspid annulus being imaged *Videographer: Please film the screen*
3.3.2. SCREEN: Lateral and septal mitral annulus being imaged *Videographer: Please film the screen* Use take 1

4. Right Heart Catheterization and Pressure Volume Loop Acquisition

4.1. For catheterization of the right heart, introduce the Swan-Ganz catheter into the jugular 8-French sheath inserted into the jugular vein **[1-TXT]** and acquire the mean right atrial, right ventricular, and pulmonary artery pressures **[2]**. *Videographer: This step is important!*

4.1.1. WIDE: Talent introducing catheter *Videographer: More Talent than pig in shot* **TEXT: See text for jugular sheath insertion details**
4.1.2. SCREEN: Shot of mean right atrial, right ventricular, and pulmonary artery pressures *Videographer: Please film the screen*

4.2. Next, measure the cardiac output with the thermodilution method according to the manufacturer's instructions **[1]** while simultaneously measuring the heart rate for the stroke volume calculation **[2]**.

4.2.1. MED: Talent measuring cardiac output *Videographer: More Talent than pig in shot* **TEXT: Use 4 °C-saline to avoid cardiac output overstimulation** injection + screen
4.2.2. CU: Heart rate being measured

4.3. Connect the disposable transducer to the pressure volume-loop work station for live acquisitions of the pressures derived from the fluid filled catheters **[1]** and use fluoroscopy to introduce the conductance catheter into the right ventricle **[2]**.

4.3.1. MED: Talent connecting transducer to PV-loop work station
4.3.2. SCREEN: Catheter being introduced into right ventricle *Videographer: Please film the screen* take 3 screen + MED

4.4. Then verify the quality signal using "in live" acquisition of the pressure-volume loops and acquire pressure volume-loop families in the steady state and during acute preload reduction during end-expiratory apnea **[1]**.

4.4.1. SCREEN: Shot of PV-loops and PV-loop famil(ies) being acquired in the steady state and/or during preload reduction *Videographer: Please film the screen* take 2, take 3 preload balloon

5. Acute Right Heart Failure Induction

5.1. To induce acute right heart failure, first use a free-flow infusion output to start a 15 milliliters/kilogram saline infusion **[1]**. *Videographer: This step is important!*

5.1.1. WIDE: Talent using free-flow infusion output to start saline infusion Videographer NOTE: slated 5.5.1

5.2. Five minutes after hemodynamic stabilization and at the end of each infusion, obtain the right heart catheterism, pressure volume-loop, and echocardiographic measurements **[1]**.

5.2.1. MED: Talent acquiring catheter measurement(s) *Videographer: More Talent than pig in shot*

5.3. Then start the second infusion of 15 milliliters/kilogram of saline immediately after the end of the measurements **[1]** and start the third infusion of 30 milliliters/kilogram of saline immediately after the end of the second set of measurements **[2]**.

5.3.1. MED: Talent loading saline for infusion
5.3.2. CU: Saline being infused Videographer NOTE: combined with 5.3.1

6. Pressure Overload with Iterative Pulmonary Embolism Induction

6.1. To induce hemodynamic pressure overload, use the fluoroscope **[1]** to insert a 5-French angiographic catheter through the jugular sheath into the right lower lobe pulmonary artery **[2]**.

6.1.1. WIDE: Talent at fluoroscope, inserting catheter *Videographer: More Talent than pig in shot*
6.1.2. SCREEN: Catheter being inserted into artery *Videographer: Please film the screen* Videographer NOTE: combined with 6.1.1, take 2
6.1.3. Added shot: Extra output rate measures

6.2. Embolize the right lower lobe pulmonary artery with a 150-microliter bolus containing N-butyl-2-cyanoacrylate lipidic contrast dye **[1-TXT]**, washing out the catheter with 10 milliliters of saline once the bolus has been delivered **[2]**. *Videographer: This step is important!*

6.2.1. CU: Bolus being administered **TEXT: See text for full bolus composition details**
6.2.2. CU: Saline being delivered to catheter Videographer NOTE: combined with 6.2.1, take 1 fluorescence, following take after add ups of bolus

6.3. Two minutes after the embolization, measure the systemic and pulmonary artery pressures to evaluate the hemodynamic response **[1]**.

6.3.1. SCREEN: Shot of systemic and pulmonary artery pressures *Videographer: Please film the screen* Videographer NOTE: see 6.2.1, use next take

6.4. Then repeat the bolus delivery every 2 minutes **[1]** until hemodynamic compromise is achieved **[2]**. *Videographer: This step is difficult and important!*

6.4.1. MED: Talent delivering bolus *Videographer: More Talent than pig in shot* Videographer NOTE: see 6.2.1, use next take
SCREEN: Shot of systemic and/or pulmonary artery pressure measurements indicating compromise *Videographer: Please film the screen* Videographer NOTE: see 6.2.1, use next take

7. Systemic Hemodynamic Restoration

7.1. After reaching hemodynamic compromise, acquire pressure volume-loop and echocardiographic measurements **[1]** before starting a dobutamine infusion at 2.5 micrograms/kilogram/minute **[2]**. *Videographer: This step is difficult and important!*

7.1.1. WIDE: Talent acquiring PV-loop measurement *Videographer: More Talent than pig in shot*
7.1.2. CU: Dobutamine being infused. This shot was not filmed

7.2. Wait 10-15 minutes for hemodynamic stabilization **[1]** before performing right heart catheterization, pressure volume-loop, and echocardiographic measurements **[2]**.

7.2.1. SCREEN: Shot of hemodynamic stabilization readout(s) (+output rate)
7.2.2. MED: Talent acquiring PV-loop measurement *Videographer: More Talent than pig in shot* Use 2nd part
7.2.3. Added shot: Extra flurorescence

7.3. When all of the measurements have been acquired, increase the dobutamine infusion dose to 5 micrograms/kilogram/minute **[1]** and repeat the right heart catheterization, pressure volume-loop, and echocardiographic measurements once hemodynamic stabilization has been achieved as just demonstrated **[2]**.

7.3.1. MED: Talent infusing dobutamine Videographer NOTE: use 7.4.1

7.3.2. SCREEN: Shot of at least one measurement No slate, check the file name
7.3.3. Added shot: Extra fluorescence
7.3.4. Added shot: Extra output rate

7.4. Then increase the dobutamine infusion dose to 7.5 micrograms/kilogram/minute **[1]**.

7.4.1. CU: Dobutamine being delivered Videographer NOTE: MED + CU

Section – Results

8. Results: Representative Acute Right Heart Failure Phenotype Analyses

8.1. Acute volume loading does not induce acute right heart failure but rather highlights the adaptive phenotype of the chronic pulmonary hypertension model **[1]**. With volume loading, the cardiac output increases **[2]** without an increase in the right atrial pressure **[3]** and with the ventriculo-arterial coupling remaining stable **[4]**.

8.1.1. LAB MEDIA: Figure 2

8.1.2. LAB MEDIA: Figure 2: *JoVE Video Editor: please show CO graph and RAP graph and emphasize VL data clusters in CO graph*

8.1.3. LAB MEDIA: Figure 2: *JoVE Video Editor: please show CO graph and RAP graph and emphasize VL data clusters in RAP graph*

8.1.4. LAB MEDIA: Figure 2: *JoVE Video Editor: please show Ees/Ea graph and emphasize VL data clusters*

8.2. Hemodynamic compromise is associated with a significant decrease in cardiac output **[1]**, stroke volume **[2]**, and ventriculo-arterial coupling, whereas right ventricle contractility remains stable. A two-fold increase in the right atrial pressure and mean pulmonary artery pressure are also typically observed **[3]**.

8.2.1. LAB MEDIA: Figure 2: *JoVE Video Editor: please show CO, SV, and Ees/Ea graphs and emphasize PE data cluster in each graph*

8.2.2. LAB MEDIA: Figure 2: *JoVE Video Editor: please show Ees graph and emphasize PE data cluster*

8.2.3. LAB MEDIA: Figure 2: *JoVE Video Editor: please show RAP and MPAP graphs and emphasize PE data cluster in each graph*

8.3. Dobutamine delivery as demonstrated restores cardiac output, stroke volume, and ventriculo-arterial coupling within the normal range **[1]**.

8.3.1. LAB MEDIA: Figure 2: *JoVE Video Editor: please show CO, SV, and Ees/Ea graphs and emphasize Dobut cluster in each graph*

8.4. Echocardiography can be used to quantify dynamic changes in right ventricle size and function throughout the experiment **[1]**.

8.4.1. LAB MEDIA: Figure 4C: no animation or *JoVE Video Editor: please sequentially add/emphasize images from left to right of figure*

8.5. Pressure volume loop analysis allows the dynamic quantification of right ventricle end-systolic elastance and ventriculo-arterial coupling **[1]**.

8.5.1. LAB MEDIA: Figure 5

8.6. In this representative study, the 2 deaths that occurred immediately after acute pulmonary embolism were associated with acute thrombosis of the right heart cavities **[1]**.

8.6.1. LAB MEDIA: Figure 3: *please emphasize dark red tissue in right of image*

8.7. After hematein, eosin, and saffron staining, right ventricular ischemic lesions, characterized by clusters of hypereosinophilic cardiomyocytes with picnotic nuclei **[1]**, can be observed in the subendocardial **[2]** and subepicardial layers of the right ventricle free-wall **[3]**.

8.7.1. LAB MEDIA: Authors: please upload the images from Figure 6A and 6B together in a new image file to your project page without the A or B labels or hexagon, rectangle, or oval emphasizers

8.7.2. LAB MEDIA: Figure 6AB: *JoVE Video Editor: please emphasize lesions as indicated with ovals in original Figure 6B*

8.7.3. LAB MEDIA: Figure 6AB: *JoVE Video Editor: please emphasize lesion as indicated with hexagon as in original Figure 6B*

Section - Conclusion

9. Conclusion Interview Statements: (Said by you on camera) - All interview statements may be edited for length and clarity.

9.1. **David Boulate**: This procedure will reveal the adaptive phenotype of the right ventricle in the context of chronic pulmonary hypertension. Therefore, magnitudes of volume and pressure overload should be adapted.

9.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 5.1 and 6.2*

9.2. **David Boulate**: The hemodynamic restoration can be performed by using other drugs or intervention. This could help to determine the best intervention in this pathological context.

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