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Closed chest biventricular pressure-volume loop recordings with admittance catheters in a porcine model

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TITLE:

Closed Chest Biventricular Pressure-Volume Loop Recordings with Admittance Catheters in a Porcine Model

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SUMMARY:

Here we present a closed chest approach to admittance-based bi-ventricular pressure-volume loop recordings in pigs with acute right ventricular dysfunction.

ABSTRACT:

Pressure-volume (PV) loop recording enables the state-of-the-art investigation of load-independent variables of ventricular performance. Uni-ventricular evaluation is often performed in preclinical research. However, the right and left ventricles exert functional interdependence due to their parallel and serial connections, encouraging simultaneous evaluation of both ventricles. Furthermore, various pharmacological interventions may affect the ventricles and their preloads and afterloads differently.

We describe our closed chest approach to admittance-based bi-ventricular PV loop recordings in a porcine model of acute right ventricular (RV) overload. We utilize minimally invasive techniques with all vascular accesses guided by ultrasound. PV catheters are positioned, under fluoroscopic guidance, to avoid thoracotomy in animals, as the closed chest approach maintains the relevant cardiopulmonary physiology. The admittance technology provides real-time PV loop recordings

without the need for post-hoc processing. Furthermore, we explain some essential troubleshooting steps during critical timepoints of the presented procedure.

The presented protocol is a reproducible and physiologically relevant approach to obtain a bi-ventricular cardiac PV loop recording in a large animal model. This can be applied to a large variety of cardiovascular animal research.

INTRODUCTION:

Pressure-volume (PV) loops contain a large number of hemodynamic information, including end-systolic and end-diastolic pressures and volumes, ejection fraction, stroke volume, and stroke work[1]. Furthermore, transient preload reduction creates a family of loops from which load-independent variables can be derived[2,3]. This load-independent evaluation of ventricular function makes PV loop recordings state-of-the-art in hemodynamic evaluation. PV loop recording can be performed in humans but is primarily used and recommended in preclinical research[4–6].

Pressure-volume loops can be obtained from both the right ventricle (RV) and the left ventricle (LV). Most research hypotheses are focused on a single ventricle, resulting in only univentricular PV loops being recorded[7–10]. However, the right and left ventricles exert systolic and diastolic interdependence due to their serial and parallel connections within the tight pericardium[11]. Changes in the output or the size of one ventricle will affect the size, loading conditions, or perfusion of the other ventricle. Thus, bi-ventricular PV loop recordings provide a more comprehensive evaluation of the total cardiac performance. Pharmacological interventions may also affect the two ventricles and their loading conditions differently, further emphasizing the importance of bi-ventricular evaluation.

PV catheters can be advanced into either ventricle by several approaches, including open chest approach with access from the apex of the heart or through the RV outflow tract[7,10,12–14]. However, the opening of the thorax will affect the physiological conditions and may introduce bias.

Based on our experience from previous studies[15–18], we aim to present our closed chest approach to bi-ventricular PV loop recordings in a large animal model of acute RV failure having minimal influence on cardiopulmonary physiology (**Figure 1**).

PROTOCOL:

This protocol was developed and utilized for studies conducted in compliance with the Danish and Institutional guidelines on animal welfare and ethics. The Danish Animal Research Inspectorate approved the study (license no. 2016-15-0201-00840). A Danish, female slaughter pig (crossbreed of Landrace, Yorkshire, and Duroc) of approximately 60 kg was used.

1. Anesthesia and ventilation

1.1. Pre-anesthetize the awake pig with Zoletil mix 1 mL/kg (see **Table of Materials**) as an intramuscular injection to reduce stress, pain, and anxiety of the animal during transportation.

1.2. Transport the animal from farm facilities to research facilities.

1.3. Establish intravenous access in an ear vein.

1.3.1. To do so, lightly tourniquet the ear to cause venous blood stasis. Disinfect the skin over a visible, straight vein with ethanol.

1.3.2. Puncture the vein with a 20 G venous catheter and release the tourniquet. Make sure to fix the access with adhesive tape to avoid displacement.

1.3.3. Flush with isotonic saline to ensure the proper positioning of the venous catheter. Observe for slight de-coloring of the vein as the saline passes.

NOTE: If a subcutaneous bulge appears, the venous catheter is in a subcutaneous position and must be removed. Consider establishing the second intravenous access as a backup.

1.4. Once anesthetized, move the animal to an operating table. Place it in a supine position.

1.5. Intubate the pig by direct laryngoscopy with a size 7 tube. Fixate the tube to the snout/head of the animal to avoid any accidental extubation. Ensure correct positioning of the tube by looking for equal thoracic movements on ventilation, stethoscopy and/or sufficient expiratory carbon dioxide.

1.6. Connect the tube to a pre-tested mechanical ventilator and start the ventilation. Use the pressure-controlled, volume-gated ventilation with a tidal volume of 8 mL/kg and low-flow ventilation. The fraction of inspired oxygen (FiO_2) can be 0.21 for normoxia or higher. Adjust the respiratory rate to target the end-tidal carbon dioxide of 5 kPa.

1.7. Start the total intravenous anesthesia by propofol 3 mg/kg/h and fentanyl 6.25 g/kg/h. Ensure sufficient anesthesia by the lack of corneal reflexes and response to a painful stimulus. Increase infusion, if necessary.

NOTE: Do not leave the animal unattended at any time until it has regained sufficient consciousness to maintain sternal recumbency (survival protocol) or has been euthanized.

1.8. Monitor the animal with a 3-lead electrocardiogram and pulse oximetry.

1.9. Measure the body temperature. If necessary, heat the animal targeting a normal porcine temperature of 38-39 ˚C.

NOTE: Hypothermia may increase the risk of arrhythmogenesis triggered by instrumentation[19].

1.10. Insert the bladder catheter (size 14) by transvaginal access and connect to a urine

sampling bag.

1.11. Depending on the research protocol and the scientific hypothesis to be investigated, consider administering heparin intravenously (5000 IE repeated every 4-6 h, if necessary) and/or amiodaron (300 mg infusion over 20 min).

NOTE: Heparinization can be performed after intravascular accesses are established. These drugs may ease the instrumentation but might bias results. Alternatively, slow saline infusion on intravenous sheaths can prevent intra-luminal thrombosis.

1.12. Use vet ointment on the eyes to prevent dryness.

2. Intravascular accesses

NOTE: Intravascular accesses are to be established in the right external jugular vein, the left external jugular vein, left carotid artery, left femoral artery, and right femoral vein. In the pig, the external jugular vein is much larger than the internal jugular vein and, therefore, easier to access. All materials required for this section are shown in **Figure 2A**.

2.1. Shave the animal at the sites of puncture for intravascular accesses.

2.2. Disinfect the skin twice with ethanol or a similar solution.

2.3. Place a sterile drape at the disinfected area with a centrally located hole in the cover.

2.4. Use an ultrasound device with a linear probe. Cover the probe with a sterile cover and use sterile gel for vascular examination.

2.5. Use a 17 G sterile venous catheter to puncture the skin and guide the needle to intravascular positioning by ultrasound (**Figure 2B,C**).

2.6. Replace the needle with a guidewire using the Seldinger technique. Next, make a small skin incision (~5 mm) adherent to the guidewire to ease the insertion of the sheath.

2.7. Remove the venous catheter leaving just the guidewire in the intravascular lumen. Place an 8 French (F) sheath over the guidewire and into the vessel of choice (the Seldinger technique). Choose an 8F sheath in the right external jugular vein (for the right heart catheterization) and the left carotid artery (for LV PV loop catheter). Sufficient lumen is necessary to avoid damaging the catheters.

2.8. Place a 7F sheath in the left external jugular vein. It will later be exchanged for a larger sheath (see steps 4.4-4.6).

2.9. Place a 7F sheath in the left femoral artery. The access is for invasive blood pressure

measurement and blood gas sampling.

2.10. Place a 12F (or 14F if available) sheath in the right femoral vein for the inferior vena cava (IVC) balloon insertion. Consider using a dilator in a two-step approach for the larger sheaths.

2.11. Confirm and control the positioning of all sheaths by drawing blood (venous or arterial, respectively) and easy flushing with isotonic saline. The sheaths are correctly positioned inside a blood vessel if one can draw blood without resistance.

2.12. Fixate all sheaths with a skin suture (size 3.0) to avoid any accidental removal of a sheath. Skin sutures will be removed after protocol completion along with the removal of sheaths.

2.13. Connect the femoral arterial access to the pressure transducer and calibrate to atmospheric pressure. Ensure this setup generates the correct form of the arterial pressure curve.

2.14. Draw an arterial blood sample from an arterial sheath and analyze it on an arterial blood sampler device to evaluate pH, arterial partial pressure of carbon dioxide ($PaCO_2$), and oxygen (PaO_2, depending on your chosen FiO_2), as well as hemoglobin, electrolytes, blood glucose, and lactate levels.

2.14.1. Correct electrolytes and blood glucose, if necessary, to the standard values by infusion of the needed product. Especially, consider the correction of potassium levels as potassium disturbances may increase the risk of arrhythmogenesis triggered by instrumentation.

2.15. If the pig was fasting prior to the experiment, consider bolus infusion of isotonic saline (10 mL/kg infused over 30-60 min) or similar crystalloid to counteract hypovolemia.

2.16. Consider a continuous infusion of 4 mL/kg/h isotonic saline to counteract perspiration throughout the protocol.

NOTE: The experiment can be paused at this step.

3. Right heart catherization

3.1 Flush a Swan Ganz catheter with saline and ensure the balloon is inflating correctly.

3.2 Connect the Swan Ganz catheter's ports to the pressure transducers. Reset the pressure to atmospheric pressure holding the two pressure ports (for pulmonary arterial and central venous pressure, respectively) at the mid-axillary level of the pig.

3.3 Insert the Swan Ganz catheter through the 8F sheath in the right jugular vein (step 2.7).

CAUTION: Lead aprons or similar protection should be worn whenever using fluoroscopy.

3.4 Observe on fluoroscopy when the distal part of the Swan Ganz catheter is out of the sheath. Inflate the balloon with the associated syringe.

NOTE: Inflation of the Swan Ganz balloon inside the sheath will damage the balloon. Anterior-posterior view of fluoroscopy is sufficient for all described procedures.

3.5 Advance the Swan Ganz catheter slowly following its movements on fluoroscopy. Slower advancements will allow the blood flow to guide the catheter.

3.6 Observe changes in the pressure signal from the distal port as it enters the RV and shortly after the pulmonary artery (**Figure 3**). Ensure that the catheter advances without any resistance.

3.6.1 Ensure that the pressure changes from 5-8 mmHg in the central venous circulation to 20-30 mmHg in systole and 0-5 mmHg in diastole in the RV. After passing the pulmonic valves, the diastolic pressure will be 10-15 mmHg (see **Figure 3** for changes in shapes of the pressure signal).

NOTE: Systolic pressures in the RV and in the pulmonary artery above 40 (or a mean pulmonary arterial pressure above 25) might be a sign of pulmonary hypertension due to pneumonic infection in the animal. Please remember that positive pressure mechanical ventilation also may increase pulmonary arterial pressure.

3.7 Deflate the balloon and ensure that the distal pressure port is still in the main pulmonary artery. Use both fluoroscopy and the pressure signal for this verification.

4. Right ventricular pressure-volume catheter insertion (Figure 4)

4.1 Read and follow the manufacturer's instructions. Allow the PV catheter to soak in saline for at least 30 min.

4.2 Open the data acquisition software (see **Table of Materials**) with an 8-channel setup (pressure, volume, phase, and magnitude from both ventricles). Click **Start** to ensure the pressure signal is recorded. Look for the excessive noise in the pressure signal. Values will be close to 0 mmHg as the pressure recorder is still outside the animal.

4.3 Calibrate the pressure to the zero-level by holding the pressure port just below the surface of saline to avoid unwanted pressure effects from the water column above.

4.4 Insert a long guidewire through the 7F sheath in the left jugular vein (step 2.7). Guided by fluoroscopy, advance the guidewire through the upper central veins, the right atrium (RA),

and the inferior vena cava. Ensure that the advancement is without any resistance. Premature systolic events are common as the guidewire passes the RA.

4.5 Extract the 7F sheath leaving the guidewire in the venous circulation. Compress the entry point to avoid bleeding. Using the Seldinger technique, exchange the 7F sheath for the 16F sheath. Extend the skin incision for the larger sheath if necessary.

4.6 Guided by fluoroscopy, advance the 16F sheath over the guidewire until the tip of the sheath (not the dilator) has reached the level of the superior vena cava (**Figure 4B**).

4.7 By carefully pulling, extract the dilator and guidewire, but be careful not to remove the sheath. Flush the sheath with isotonic saline to avoid blood clotting.

4.8 Insert the PV catheter in the 16F sheath.

4.9 Use fluoroscopy to follow the PV catheter as it passes through the sheath until the pressure-port has left the sheath.

4.10 Carefully advance the sheath and PV catheter collectively until the sheath is just outside the pericardial border.

4.11 Advance the PV catheter into the RA (**Figure 4C**).

4.12 Use the sheath length to help advance the PV catheter from the RA into the more anteriorly positioned RV; point the external end of the 16F sheath downwards (posterior to the supine animal) and medially, which will point the internal end of the sheath anteriorly.

4.13 Advance the PV catheter into the RV. This can be verified by the change in pressure-signal from the PV catheter to a classic ventricular shape and by the tactile resistance as the PV catheter meets the right ventricular apex.

4.14 Once the PV catheter is in the RV, retract the 16F sheath outside the thoracic cavity to avoid any hemodynamic or electrical influence of the device located close to the heart (**Figure 4D**).

4.15 Optimize the PV catheter positioning, based on fluoroscopy, as close to the RV apex as possible, but do not let it touch the endocardium.

NOTE: Use fluoroscopy to observe the excess mechanical contact between PV catheter and endocardium, if any. This is viewed as a bended PV catheter (including its pigtail) and persistent premature systolic events via the electrocardiographic monitoring.

4.15.1. Fixate the PV catheter to the external end of the sheath with adhesion tape to ensure the stability of catheter positioning.

NOTE: Occasionally, a floating catheter may cause extra-beats. If so, try fixating it without compressing the endocardium too much.

4.16 Follow the manufacturer's protocol to choose the relevant number of recording segments and to optimize the PV catheter positioning in the RV, based on the recorded phase and magnitude signals.

NOTE: For pigs weighing 60 kg, two or three segments for the RV and most often three segments for the LV were used for this experiment. Fewer segments will be required in smaller animals and vice versa. Positioning of the catheter was based on the magnitude of signals initially; the shape of the pressure-magnitude loop should look like the desired pressure-volume loop. The magnitude amplitude should be as high as possible (5-10 mS). The phase angle should be within 1-3 ^{o} with the highest possible amplitude (approximately 1.5 ^{o}).

5. Left ventricular pressure-volume catheter insertion (Figure 5)

5.1. Read and follow the manufacturer's instructions. Allow the PV catheter to soak in saline for at least 30 min.

5.2. Calibrate the pressure to zero-level (step 4.3).

5.3. Insert the PV catheter in the 8F sheath in the left carotid artery.

5.4. Follow the PV catheter by fluoroscopy as it passes through the sheath towards the aortic valves (**Figure 5B**). A resistance is felt when the PV catheter is stopped by the aortic valves. On fluoroscopy, bending of the catheter is observed.

NOTE: Occasionally, the PV catheter turns into the descending aorta. This is recognized by fluoroscopy, and a less prominent aortic notch on the pressure-curve of the PV catheter.

5.5. Retract the PV catheters approximately 1 cm above the aortic valves.

5.6. Synchronize the next quick advancement of the PV catheter to a systolic phase of the cardiac cycle. This will happen through the open aortic valves. Success can be verified by the change in the pressure signal from the PV catheter to a classic ventricular shape.

5.7. If attempts to advance through the valves fail, rotate the PV catheter for better positioning in the center of the ascending aorta. Retry, if needed.

5.8. Once inside the LV, optimize the left ventricular PV catheter positioning based on fluoroscopy, as close to the LV apex as possible, but do not let it touch the endocardium (**Figure 5C**). See step 4.15.

NOTE: Occasionally, a floating catheter may cause premature cardiac contractions. If so, try fixating it without compressing the endocardium too much.

5.9. Follow manufacturer's protocol to choose the relevant number of recording segments and to optimize the PV catheter positioning in the LV, based on the recorded phase and magnitude signals.

6. Inferior vena cava balloon insertion

6.1. Fill the syringe for inflation with saline or contrast agent as preferred and ensure that the balloon can be inflated correctly.

6.2. Insert the guidewire in the 12F sheath in the right femoral vein.

6.3. Advance the guidewire to the IVC at the level of the diaphragm.

6.4. Insert the balloon over the guidewire and advance it to the diaphragm level at the end expiration (**Figure 5D**).

6.5. Retract the guidewire and flush the lumen with saline to avoid blood clotting.

7. Pressure-volume catheter calibration

7.1. Read and follow the manufacturer's instructions.

7.2. Ensure stable sinus rhythm on the electrocardiographic monitor and stable cardiopulmonary variables for 5-10 min.

7.3. Use the Swan Ganz catheter to measure the cardiac output (CO) by thermodilution. Use an average of three injections of 10 mL of 5 ˚C isotonic glucose with less than 10% variation. Observe the animal's heart rate (HR) during the CO measurement. Calculate the stroke volume (SV) as SV = CO/HR (unit mL). Normal CO is 4-6 L/min for a 60 kg pig with a stroke volume of 80-110 mL.

7.4. Enter the SV into the PV boxes for both the LV and RV.

7.5. Check that optimal phase and magnitude signals are received from both ventricles. Notably, the two PV boxes must record at different frequencies to avoid electronic cross-talking.

7.6. In transient apnea, calibrate ("scan") the PV signals.

7.7. If calibration is satisfactory, ensure the proper shape of both ventricular PV loops, as well as realistic pressures and volumes. If not, re-do the calibration.

8. Baseline evaluation

NOTE: Experiment can be paused at this level for the stabilization of hemodynamics before the research protocol begins.

8.1 When PV loops are to be recorded, follow the manufacturer's instructions. Press **Start** in the data acquisition software. Ensure the PV loops are still acceptably shaped.

8.2 Record PV loops over 30-60 s of continuous ventilation. Perform analysis by finding the average of e.g., three respiratory cycles. Alternatively, perform a transient breath-hold for the end expiration on the ventilator and analyze these loops from apnea. Consider having low/no positive end-expiratory pressure (PEEP) and minimal adjustable pressure limiting (APL) valve.

NOTE: Ventricular function, especially the RV, is affected by cyclic changes of intrathoracic pressures during ventilation (or spontaneous respiration). Importantly, report in the paper if PV loops were recorded during ventilation or in apnea.

8.3 For load-independent PV variables, do a breath-hold and wait a few heart beats before slowly inflating the IVC balloon with the chosen liquid (step 6.1). The balloon progressively decreases the cardiac preload.

8.4 Observe how the RV PV loops become progressively smaller and leftward shifted.

NOTE: The gradual decrease in RV preload will lower RV end diastolic volume progressively. Lower volumes will cause lower pressures and output (Starling mechanism). For further details, see references[1-3].

8.5 Importantly, keep the balloon inflated by keeping the pressure on the associated syringe long enough for the reduction in LV preload (serially connected with the RV). Observe progressive decrease in LV pressure and volume as well. See the Representative Results section for examples.

8.6 Quickly, deflate the balloon and turn on ventilation.

8.7 Re-do 8.3-8.7 if the response was not satisfactory, i.e., without premature cardiac complexes, sinus bradycardia, or similarly affected cardiac function.

8.8 Allow the pig to stabilize for 2-5 min before the next IVC occlusion.

NOTE: Hemodynamics are transiently affected by the breath-hold and preload reduction, especially in models of cardiovascular impairment.

8.9 Consider performing three satisfactory occlusions (see 8.7) to increase the robustness of

the statistical analyses.

9. Post Protocol

9.1 In survival studies, remove and clean all intravascular equipment (PV catheters, IVC balloon, and Swan Ganz catheter).

9.1.1 Cut the skin sutures that kept the sheaths in place. Remove each sheath by manual pulling. Compress on each venous access site for a few minutes to achieve hemostasis.

9.1.2 For arteries, remove the sheath and compress longer (5-10 min) to achieve hemostasis. Alternatively, consider using a vascular closure device.

9.1.3 Close the skin incisions from the sheaths with one adaptive skin suture (3.0, absorbable suture) to avoid bleeding and infection. Apply 5 mL of bupivacaine (5 mg/mL) subcutaneously around each skin incision for pain relief.

9.2 Once all devices have been removed and hemostasis is achieved, stop the infusion of anesthesia. Carefully observe the animal in this phase.

9.3 Keep the animal intubated (initially with the cuff inflated) until throat reflexes are present and the animal is sufficiently awake for extubation. Keep measuring the oxygen levels via pulse oximetry before and after extubation to ensure proper ventilation. Apply oxygen if necessary.

9.4 Do not return the animal to the company of other animals until fully recovered.

9.5 For survival surgery, maintain proper sterile conditions. Please see steps 2.2-2.5. Observe the skin incisions and sutures daily for signs of infection including measurement of the animal's temperature.

9.6 Once the experiment ends, perform euthanasia with a lethal dose of pentobarbital (15 mL, 400 mg/mL).

REPRESENTATIVE RESULTS:

The present instructions describe an approach to achieve admittance-based PV recordings from both the RV and the LV in a large animal.

To compare our simultaneous PV recordings in the RV and LV, we performed a linear regression of the bi-ventricular CO measurements from our largest study[18] with the highest number of simultaneous RV CO and LV CO measurements (n=379 recordings from 12 animals). We found that the slope was 1.03 (95%CI 0.90-1.15) with a Y-intercept of 695 (95%CI -2-1392) and r^2=0.40. This suggests a good correlation between CO measured by the PV catheter in each ventricle.

Figure 6 shows PV loops from the RV and LV and represents both acceptable loops (**Figure 6A,B**), as well as suboptimal loops (**Figure 6C,D**). Loops are not from the same animal but chosen for representative reasons. The investigator should pay close attention to the shape of the loops and adjust the PV catheters to improve the quality of loops (see manufacturer's instructions). Usually, sufficient PV loops can be easily obtained from the LV; the investigator should always aim for classic squared loops. In the RV, it is occasionally more difficult to get classic triangular loops without noise. Some static noise (**Figure 6D,** lower right corner of the loop) from blood turbulence in the end-diastole is acceptable.

The serial connection of the two ventricles causes a timewise shift in preload reduction (see section 8.6). IVC balloon quickly reduces RV preload, but LV preload is not reduced until RV output has decreased by its lack of preload, see **Figure 7A**. In each single animal, gradual reduction in the preload will cause a family of loops with gradual reduction in volume and pressure to both the LV and RV (**Figure 7B,C**). Load-independent variables from these families of loops are analyzed by the data acquisition software. The end-systolic pressure-volume relationship corresponds to the end-systolic elastance (ventricular contractility). Preload-recruitable stroke work (PRSW) is another variable of contractility correlating ventricular stroke work to end-diastolic volume. The end-diastolic pressure-volume relationship corresponds to end-diastolic elastance and is a measure of ventricular diastolic function. All correlations were obtained with the data acquisition software during post-protocol analyses.

Please note that only load-independent variables are obtained from the family of loops by preload reduction. "Standard" PV variables (e.g., volumes, pressures, ejection fraction, first derivatives of pressure etc.) are obtained from the recordings during ventilation and normal preload (step 8.2). These are again analyzed and delivered by the data acquisition software.
All variables should be analyzed with the observer blinded.

By following this protocol, it is possible to record real-time PV loops from both ventricles simultaneously. These recordings can detect effects on both ventricles from a disease model[17,18] as well as changes from interventions targeting preload[15] and afterload[16,17].

FIGURE AND TABLE LEGENDS:

Figure 1: Instrumentation overview. The pig is anesthetized, mechanically ventilated and in supine position. (**A**) illustrates a sheath in the right external jugular vein through which a Swan Ganz catheter is advanced to the pulmonary artery. (**B**) shows the left ventricular pressure-volume catheter inserted through the left carotid artery, where (**C**) is the right ventricular pressure volume catheter inserted through the left external jugular vein. From the right femoral vein, an inferior vena cava balloon is advanced to the diaphragmatic level (**D**). Compare this to the fluoroscopic picture, Figure 5D.

Figure 2: Intravascular access guided by ultrasound. (**A**) Ensure all equipment is ready, sterile, and well-functioning. Necessary equipment include 7F sheaths (orange), 8F sheaths (blue) and a 12F sheath (white), guidewires for the Seldinger technique, venous catheters for intravascular

access, syringe, isotonic saline, scalpel and suture. (**B**) Use a linear ultrasound probe to guide the insertion of a venous catheter to the requested vessel. The tip of the needle should always be followed to avoid puncturing the surrounding tissue. At (**C**), the needle (white arrow) is placed centrally in the femoral vein (partly marked with dashed blue) using the out-of-plane ultrasound approach. The femoral artery is partly marked with dashed red and should be spared for punctuation using the ultrasound-guided technique. Avoidance of cut-down technique minimizes traumatic, pain, and stress responses in the animal.

Figure 3: Right heart catherization. Equipment is shown in (**A**) with a Swan Ganz catheter (yellow arrow) and a syringe and isotonic saline. Ensure the tip balloon is working properly. Fluoroscopic pictures are shown in (**B-D**). The Swan Ganz catheter is advanced with an inflated balloon (the halo around the tip of the catheter, marked with a dashed arrow). The Swan Ganz catheter passes the right atrium (**B**), the right ventricle (**C**, anterior direction i.e., out of the picture) and into the pulmonary artery (**D**). Ensure the tip does not retract to the right ventricle when the balloon is deflated. The balloon must be deflated ultimately (**D**, no halo) to avoid compromising blood flow or cause wedging. Please note, that in these pictures the Swan Ganz catheter is advanced through a large sheath as pictures stem from our model of right ventricular failure (reference 18) where the large sheath is used for pulmonary embolism induction. The large sheath itself is not necessary for the closed chest bi-ventricular pressure-volume instrumentation presented here and therefore, not included in the present protocol.

Figure 4: Right ventricular pressure-volume catheter insertion. Materials needed are shown in (**A**) and includes the pressure-volume catheter (blue arrow), a guidewire and the 16F 30 cm sheath (black arrow). (**B**) shows a fluoroscopic picture of the 16F sheath advanced over a guidewire which continues into the inferior vena cava. Advance the pressure-volume catheter through the sheath into the right atrium (**C**). Use the length of the sheath to aim its tip towards the right ventricle and advance the pressure-volume catheter. Note the different pressure-signals outside versus inside the right ventricle. Ultimately, retract the sheath out of the thoracic cavity (**D**).

Figure 5: Left ventricular pressure-volume catheter and inferior vena cava insertions. Materials needed are shown in (**A**) and includes pressure-volume catheter (red arrow) and inferior vena cava balloon (green arrow). The left ventricular pressure-volume catheter is advanced retrogradely (from the top in the picture) with an aortic pressure signal (**B**). After passing the aortic valves, the pressure-signal changes and the catheter can be placed close to the apex (**C**). The inferior vena cava balloon is advanced from the inferior to the level of the diaphragm (**D**). The part of the diaphragm is marked with a dashed green curve. The balloon must be deflated when advanced and positioned and only transiently inflated when load-independent pressure-volume variables are recorded. Compare this panel with the overview of the instrumentation in Figure 1.

Figure 6: Variety of pressure-volume loops from both ventricles. To the left, pressure-volume loops from the left ventricle are shown. (**A**) is an optimal squared loop, classic for the left ventricle, whereas (**C**) is a suboptimal loop. The latter should be improved as it is usually

possible to get good loops from the left ventricle. To the right, pressure-volume loops from the right ventricle are shown. (**B**) is an optimal loop without noise and has a triangular shape. (**D**) represent loops with more noise, often seen in the lower right corner i.e., at the end-diastole where blood flow changes direction in the ventricle which causes turbulence.

Figure 7: Preload reduction by inferior vena cava balloon inflation. (**A**) shows simultaneous recordings of pressure, volume, phase, and magnitude from the left ventricle (top) and the right ventricle (bottom). X-axis is time. Please note, how pressure and volume is reduced in the right ventricle prior to the reduction in the left ventricular pressure and volume. Accordingly, the inferior vena cava balloon must be inflated long enough to cause the preload reduction in both ventricles (steps 8.4-8.6). (**B**) and (**C**) shows a representative family of pressure-volume loops (i.e., volume on the x-axis and pressure on the y-axis) during such preload reduction for the left ventricle (**B**) and the right ventricle (**C**).

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DISCUSSION:

This paper describes a reproducible minimally invasive closed chest approach for bi-ventricular pressure-volume loop recordings.

Advancement of the PV catheter from the RA into the RV is the most critical step in this protocol. The complex composition of the RV and the stiffness of the catheter complicate insertion into the easily distended and geometrically challenging RV. This difficulty may explain why open chest instrumentation is often preferred. During pilot studies, numerous accesses and techniques were tried and discarded, including right external jugular vein access, suprasternal access into the superior vena cava, and from the inferior vena cava. Based on these pilot studies, access from the left side of the neck was found to be the easiest and most reproducible approach.

We aim to provide recommendations for troubleshooting this challenging step of entering the RV. First, the PV catheter will often go from the RA into the inferior vena cava. This is easily recognized by fluoroscopy when the PV catheter leaves the pericardial shadow, and no change is observed in the appropriate pressure-curve. We recommend closely observing the path of the Swan Ganz catheter through the RA to mimic the same path for the RV PV catheter. Retract the PV catheter to the top of the RA and rotate 45-180^{o} in either direction and/or manipulate the position and direction of the sheath. Occasionally, it may be necessary to advance the tip of the sheath into the RA. Innately, this is a “hit-or-miss” approach but fluoroscopic guidance is of great assistance. The same approach of the PV catheter rotation can be beneficial when encountering difficulties advancing the LV PV catheter through the aortic valves.

Rarely, the RV PV catheter has difficulty advancing to the RV despite several attempts and optimized working conditions through aforementioned troubleshooting. We use the following as a back-up approach. ompletely retract the PV catheter out of the animal. Insert another Swan Ganz catheter through the sheath in the left external jugular vein and advance it into the pulmonary artery (i.e., repeat steps 3.1-3.8, but from the left side). Use this second Swan Ganz catheter as a guidewire and advance the 16F sheath into the RV. This may cause ventricular arrhythmias, so it is advised to quickly extract the Swan Ganz catheter entirely and insert the PV

catheter through the 16F sheath directly into the RV. Retract the 16F sheath, while ensuring that the PV catheter remains in the RV. This technique puts a larger but transient mechanical strain on the heart but is efficient as a back-up technique. Alternatively, steerable sheaths can be used.

The presented approach to closed chest instrumentation of bi-ventricular PV catheters has potential significance. Previous large animal studies have often relied on univentricular PV measurement[8,20,21] These measurement have inherent shortcomings in evaluating the complete cardiovascular physiology as it may miss the interventional effect on the other ventricle. Similarly, an open chest approach is frequent in research using PV loops in large animal models[7,10,13,14,22]. However, opening of the thorax and pericardium will affect hemodynamics, especially for the RV[23,24], and may bias the results. Our techniques ensure a thorough cardiopulmonary investigation with insignificant effects on hemodynamics, thereby less risk of bias.

We used admittance-based technology for PV loop recordings. PV loops have traditionally been recorded based on the conductance technology. The newly emerged admittance-based technology allows real-time subtraction of parallel conductance, thereby avoids post-hoc processing of PV data[25]. Admittance-based PV loop recordings have been well validated[8,26].

The presented approach may not be limited to animal models of acute RV dysfunction[15–18] but can be applied in a large spectrum of cardiopulmonary research. The two ventricles are interdependent in systole as well as diastole[11,27]. The LV and septum accounts for 20-40% of RV ejection[28], and RV function is a significant predictor of outcome in LV diseases[29,30]. Therefore, we suggest that researchers performing any kind of cardiopulmonary preclinical research should consider a bi-ventricular cardiac evaluation.

The presented setup has some limitations. First, instrumentation and hemodynamic evaluation require the animal to be anesthetized and mechanically ventilated. This will vary from the normal physiology, but it is a shortcoming regardless of PV instrumentation approach. Secondly, the instrumentation requires fluoroscopy which demands attention due to the radiation exposure to the researchers. Furthermore, not all animal research facilities may have access to this specialized and expensive equipment. Thirdly, the shape of the RV is not optimal for assessing volumetry by a straight catheter, and minor parts of the RV outflow tract might be missed with our antegrade approach. However, repeated measurements performed before and/or after interventions with a fixated catheter will limit this bias. Also, PV loop recordings in general offer a number of hemodynamic variables outweighing this concern. Lastly, the instrumentation techniques might be difficult to learn compared to an open chest approach where manual manipulation of the equipment is possible.

In conclusion, we present a reproducible and physiologically relevant approach to perform bi-ventricular cardiac PV loop recordings in a large animal model. This technique may be applicable to a broad variety of cardiovascular research in large animal models.

ACKNOWLEDGMENTS:
This work was supported by the Laerdal Foundation for Acute Medicine (3374), Holger and Ruth

Hesse's Memorial Foundation, Søster and Verner Lippert's Foundation, Novo Nordisk Foundation (NNF16OC0023244, NFF17CO0024868), and Alfred Benzon's Foundation.

DISCLOSURES:

None of the authors has any conflicts of interest to declare.

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Figure 1

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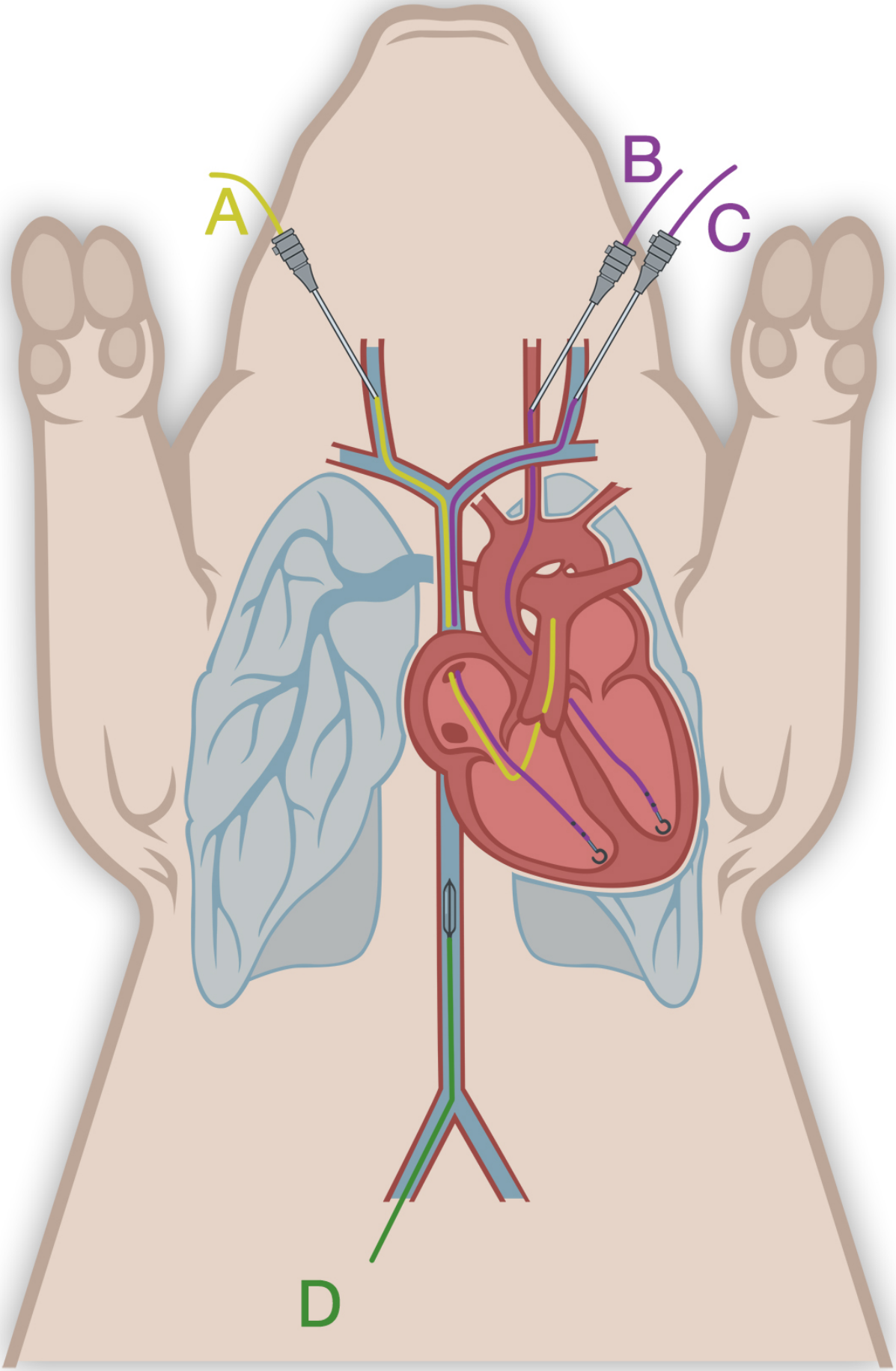

Figure 2

Figure 3

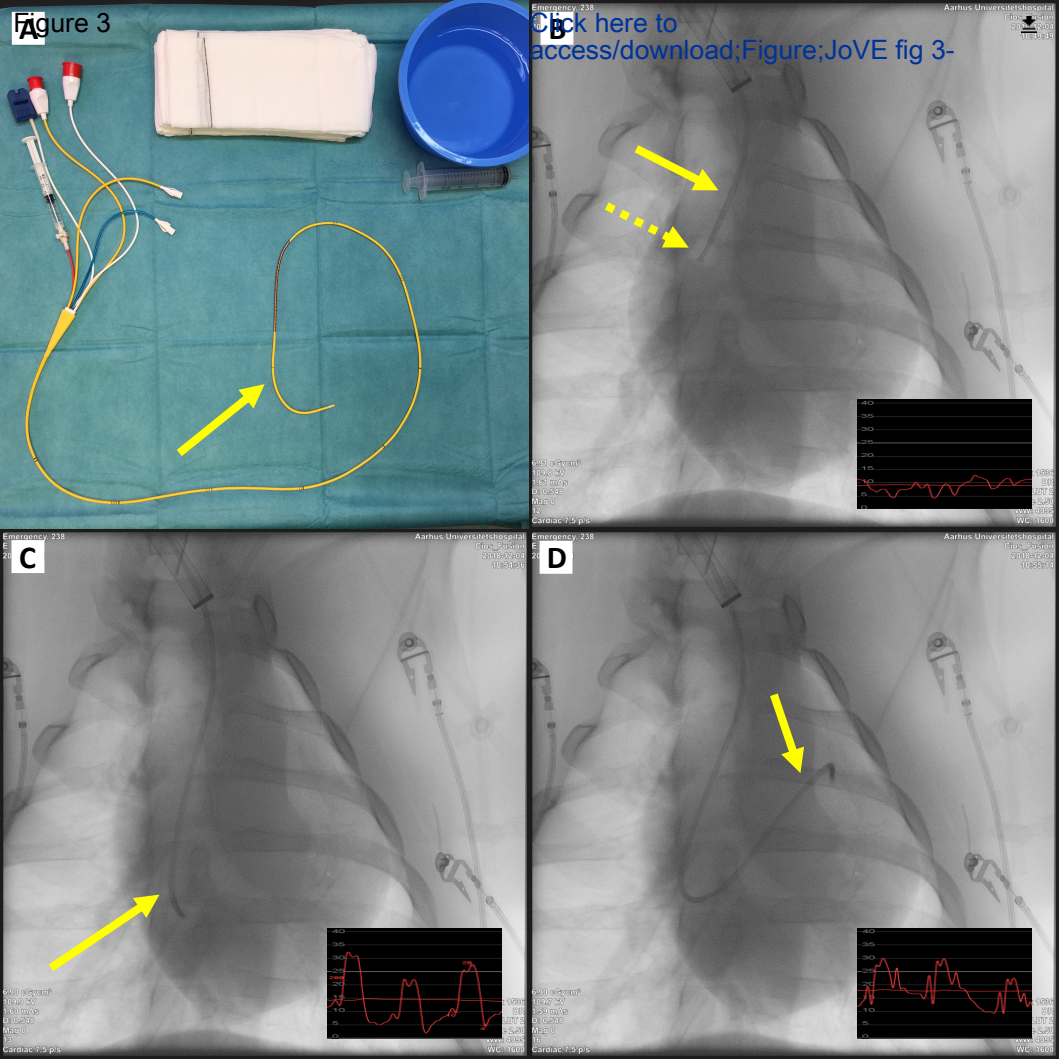

Figure 4

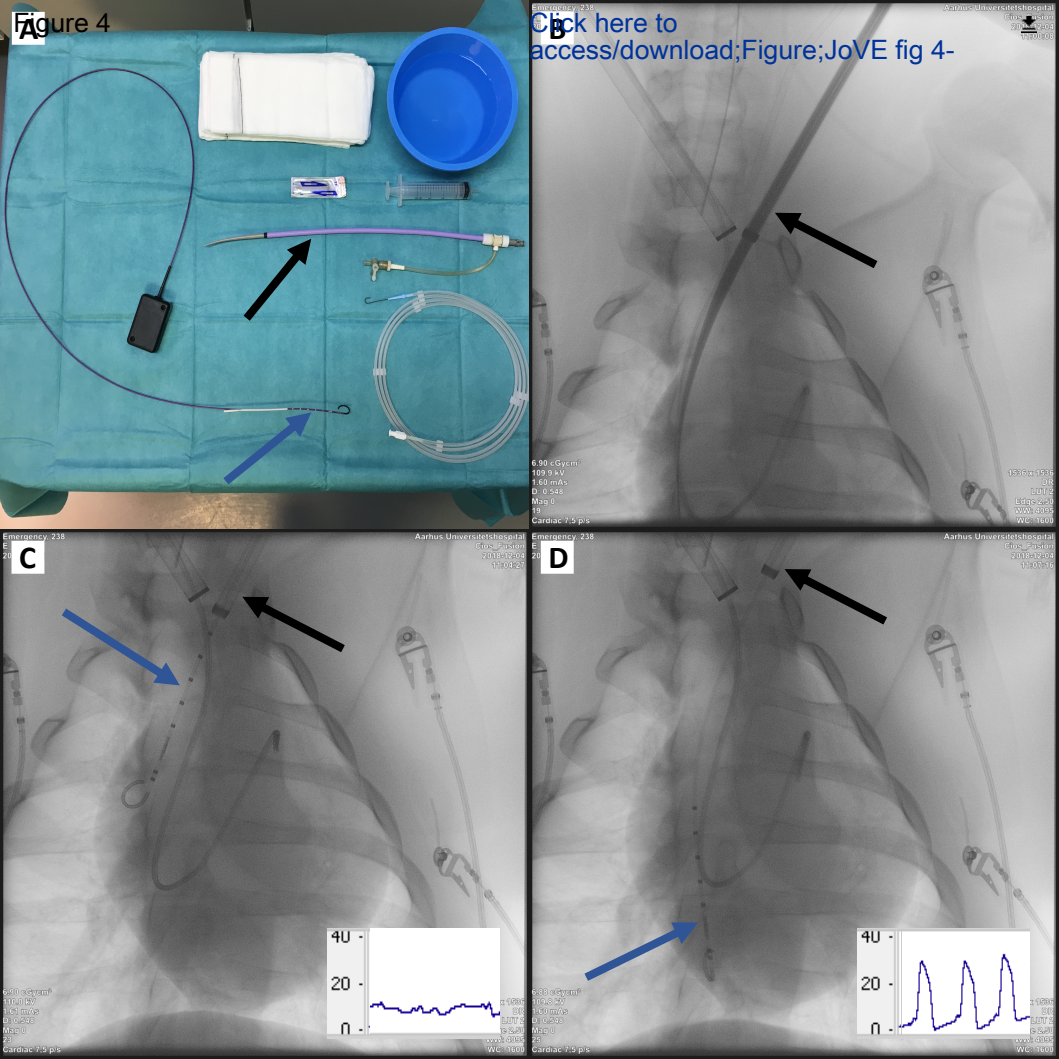
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Figure 5

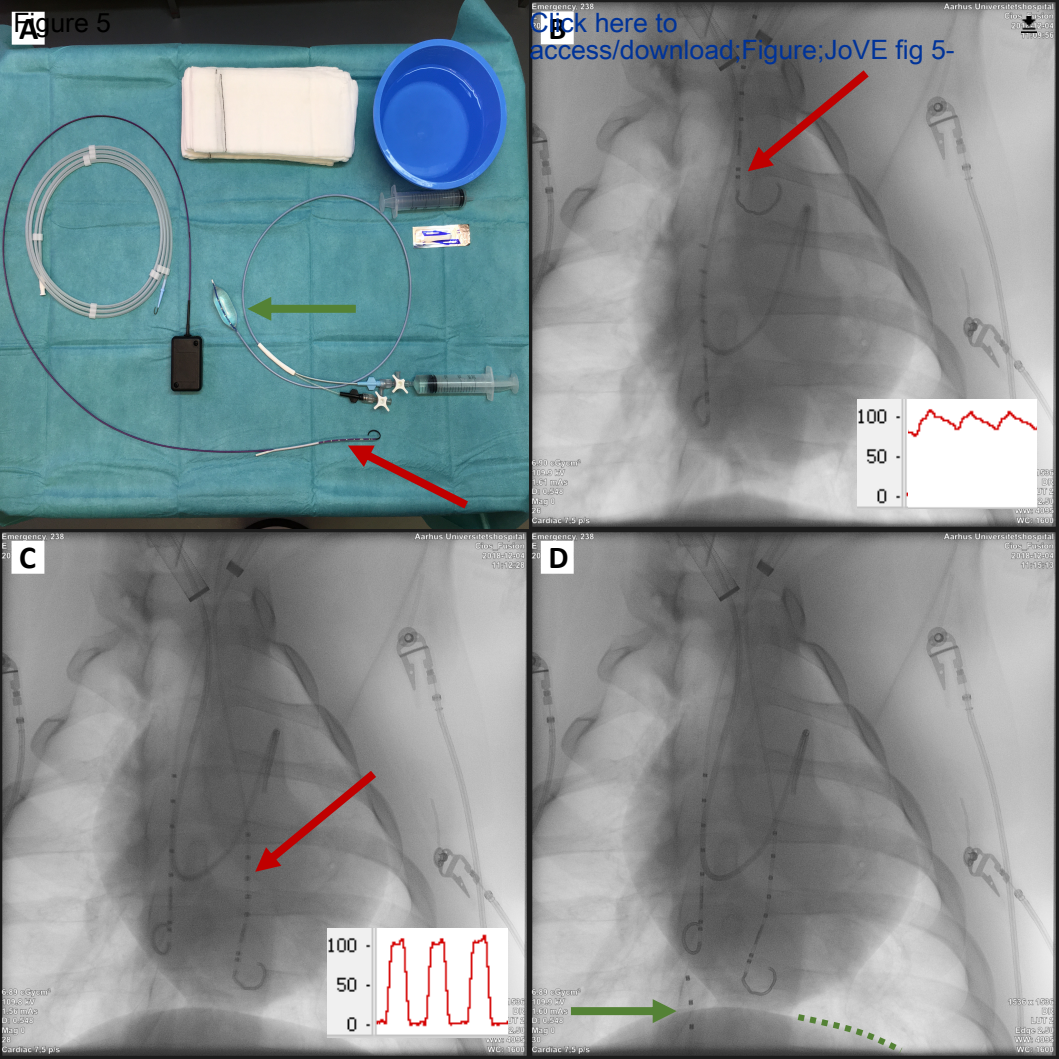

Figure 6

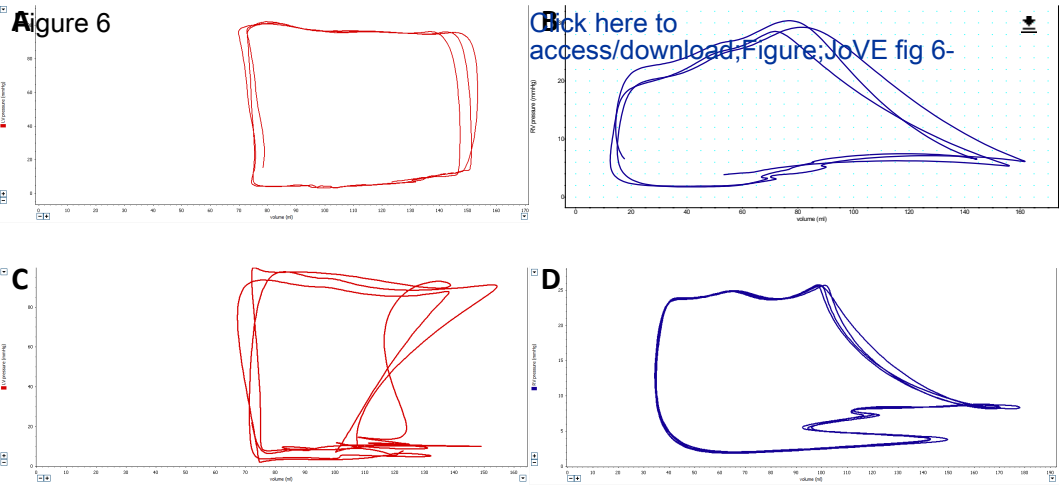

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Figure 7

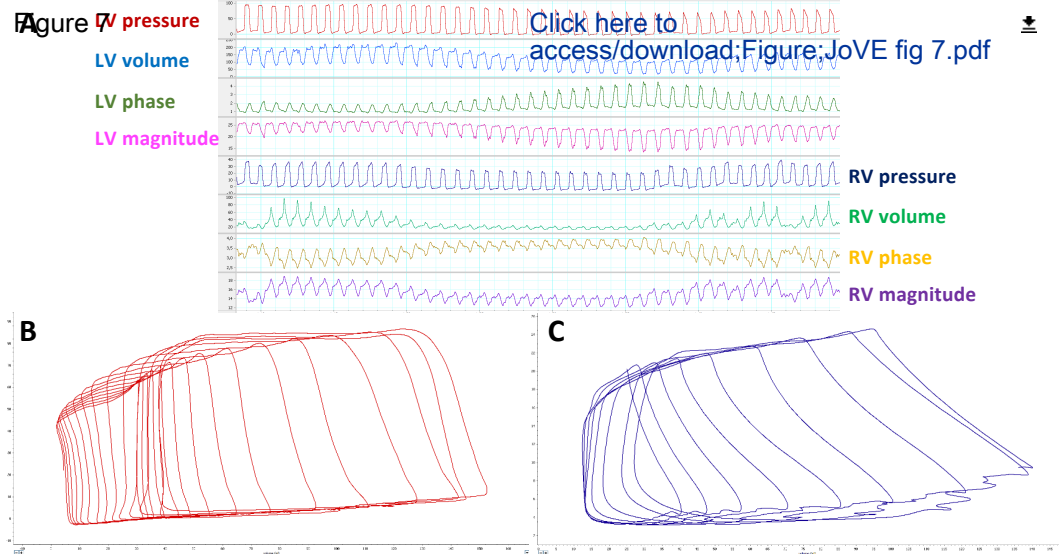

Table of Materials

Click here to access/download;Table of Materials;JoVE_Materials_Mads Dam Lyhne revised.xls

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
12L-RS	GE Healthcare Japan	5141337	Ultrasound probe
Adhesive Aperature Drape (OneMed)	evercare	1515-01	75 x 90 cm (hole: 6 x 8 cm)
Alaris GP Guardrails plus	CareFusion	9002TIG01-G	Infusion pump
Alaris Infusion set	BD Plastipak	60593	
Alkoholswap	MEDIQ Danmark	3340012	82% ethanol, 0,5% chlorhexidin, skin disinfection
Amplatz Support Wire Guide Extra-Stiff	Cook Medical	THSF-25-260-AES	diameter: 0.025 inches, length: 260 cm
BD Connecta	BD	394601	Luer-Lock
BD Emerald	BD	307736	10 mL syringe
BD Luer-Lock	BD Plastipak	300865	BD = Becton Dickinson, 50 mL syringe
BD Platipak	BD	300613	20 mL syringe
BD Venflon Pro	Becton Dickinson Infusion Therapy	393204	20G
BD Venflon Pro	Becton Dickinson Infusion Therapy	393208	17G
Butomidor Vet	Richter Pharma AG	531943	10 mg/mL
Check-Flo Performer Introducer	Cook Medical	RCFW-16.0P-38-30-RB	16 F sheath, 30 cm long
Cios Connect S/N 20015	Siemens Healthineers		C-arm
D-LCC12A-01	GE Healthcare Finland		Pressure measurement monitor
Durapore	3M	-	Adhesive tape
E-PRESTIN-00	GE Healthcare Finland	6152932	Respirator tubes
Exagon vet	Richter Pharma AG	427931	400 mg/mL
Fast-Cath Hemostasis Introducer 12F	St. Jude Medical	406128	L: 12 cm
Favorita II	Aesculap		Type: GT104
Fentanyl	B. Braun	71036	50 mikrogram/mL
Ketaminol Vet	MSD/Intervet International B.V.	511519	100 mg/mL

LabChart	ADInstruments		Data aquisition software
Lawton 85-0010 ZK1	Lawton		Laryngoscope
Lectospiral	VYGON	1159.90	400 cm (Luer-LOCK)
Lubrithal eye gel	Dechra, Great Britain		
MBH qufora	MBH-International A/S	13853401	Urine bag
Natriumklorid	Fresenius Kabi	7340022100528	9 mg/ml Isotonic saline
PICO50 Aterial Blood Sampler	Radiometer	956-552	2 mL
Portex Tracheal Tube	Smiths Medical	100/150/075	"Cuffed Clear Oral/Nasal Murphy Eye"
PowerLab 16/35	ADInstruments	PL3516	Serial number: 3516-1841
Pressure Extension set	CODAN	714,020	Tube for anesthetics, 150 cm long, inner diameter 0.9 mm
Propolipid	Fresenius Kabi	21636	Propofol, 10 mg/mL
PTS-X	NuMED Canada Inc.	PTSX253	Inferior vena cava balloon
Radiofocus Introducer II	Radiofocus/Terumo	RS+B80N10MQ	6+7+8F sheaths
Rompun Vet	Beyer	86450917	Xylazin, 20 mg/mL
Rüsch Brilliant AquaFlate Glycerine	Teleflex	178000	Bladder catheter, size 14
S/5 Avance	Datex-Ohmeda	-	Mechanical ventilator
Safersonic Conti Plus & Safergel	SECMA medical innovation	SAF.612.18120.WG .SEC	18 x 120 cm (Safersonic Sterile Transducer Cover with Adhesive Area and Safergel)
Scisense Catheter	Transonic Scisense	FDH-5018B-E245B	Serial number: 50-533. Pressure-volume catheter
Scisense Pressure-Volume Measurement System	Transonic Scisense	ADV500	Model: FY097B. Pressure-volume box
Swan-Ganz CCOmbo	Edwards Lifesciences	744F75	110 cm
TruWave Pressure Monitoring Set	Edwards Lifesciences	T434303A	210 cm
Vivid iq	GE Medical Systems China	Vivid iq	

Zoletil 50 Vet (tiletamin 125 mg and zolazepam 125 mg)	Virbac	83046805	Zoletil Mix for pigs: 1 vial of Zoletil 50 Vet (dry matter); add 6.25 mL Xylozin (20 mg/mL), 1.25 mL ketamin (100 mg/mL) and 2.5 mL Butorphanol (10 mg/mL). Dose for pre-anesthesia: 10 mL/10 kg as intramuscular injection

Letter of rebuttal
Regarding manuscript JoVE62661

To the editorial office of JoVE,
Att. Vidhya Iyer

Thank you for considering our manuscript. We are delighted to see that our manuscript has been well received and has been through a thorough review process of three external reviewers. We appreciate their insightful comments and have tried to fulfil their request.
Below, you will find all comments from the editorial office and the reviewers. Each point will be addressed **in blue** and any changes in the manuscript are *in italics*.

We hope that the manuscript is now acceptable for publication in JoVE.

On behalf of all authors,
Yours sincerely,
Mads Dam Lyhne, MD and
Asger Andersen, MD, PhD, ass. professor

Editorial comments:
Changes to be made by the Author(s):
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. Please define all abbreviations at first use.
Comment #1: We have carefully read the manuscript and made few, minor changes.

2. Please increase the word count of your abstract to be 150-300 words.
Comment #2: The following has been added to the abstract and the word count is now 172.
"Furthermore, we explain some important troubleshooting steps during critical timepoints of the procedures". And
"The presented closed chest approach is *a reproducible and physiologically relevant approach to bi-ventricular cardiac PV loop recording in a large animal model. It will be* applicable to a large variety of cardiovascular animal research".

3. Being a video based journal, JoVE authors must be very specific when it comes to the humane treatment of animals. Regarding animal treatment in the protocol, please add the following information to the text:
a) What happened to the pig? Please specify the euthanasia method without highlighting it.
Comment #3: A "Post-protocol" section no. 9 has been added to the manuscript.
9. *Post-protocol*

9.1 In survival studies, all intravascular equipment (PV catheters, IVC balloon and Swan Ganz catheter) can easily be removed and cleaned. Sheaths in veins can be removed and hemostasis achieved by few minutes of compression. For arteries, hemostasis can be achieved by longer compression (5-10 minutes), alternatively one can consider to use AngioSeal or similar closure device. Close skin incisions with 1-2 sutures to avoid bleeding and infection. Apply 5 mL bupivacaine (5 mg/mL) subcutaneously around each skin incision for pain relief.

9.2 After all devices have been removed and hemostasis achieved, stop infusion of anesthesia. Carefully observe the animal in this phase. Keep the animal intubated (initially with the cuff inflated) until throat reflex is present and the animal is sufficiently awake for extubation. Keep measuring pulse oximetry before and after extubation to ensure proper ventilation and apply oxygen if necessary.

9.3 The animal should not be returned to the company of other animals until fully recovered.

9.4 For survival surgery, sterile conditions are crucial. Please see section 2.2-2.5. The skin incisions and sutures should be observed daily for signs of infection including measurement of the animal's temperature.

9.5 Euthanasia after ended experimental protocol was performed by a lethal dose of pentobarbital.

b) Please mention how proper anesthetization is confirmed.

Comment #4: This was stated in section 1.7. We have clarified this part.

"Ensure sufficient anesthesia by the lack of *corneal* reflexes *and response to painful stimulus*"

c) Please specify the use of vet ointment on eyes to prevent dryness while under anesthesia.

Comment #5: This has been added as point 1.12 and the product added to the Materials list.

1.12 Use vet ointment on eyes to prevent dryness.

e) For survival strategies, discuss post-surgical treatment of animal, including recovery conditions and treatment for post-surgical pain.

Comment #6: Please see Comment #3.

f) Discuss maintenance of sterile conditions during survival surgery.

Comment #7: Sterile conditions are described in section 2.2-2-5. Furthermore, comments for survival surgery has been added, please see Comment #3.

g) Please specify that the animal is not left unattended until it has regained sufficient consciousness to maintain sternal recumbency.

Comment #8: We agree on this important aspect. The following has been added to section 1.7:

"*Do not leave the animal unattended at any time until it has regained sufficient consciousness to maintain sternal recumbency (survival protocol) or has been euthanized*."

h) Please specify that the animal that has undergone surgery is not returned to the company of other animals until fully recovered.

Comment #9: Please see Comment #3.

4. Please revise the text, especially in the protocol, to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

Comment #10: The manuscript has been revised.

5. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (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.” However, notes should be concise and used sparingly. Please include all safety procedures and use of hoods, etc.

Comment #11: The manuscript has been revised and changes made accordingly.

6. Please note that your protocol will be used to generate the script for the video and must contain everything that you would like shown in the video. Please add more details to your protocol steps. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. Please add more specific details (e.g., button clicks for software actions, numerical values for settings, etc) to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.

Comment #12: The manuscript has been revised and changes made accordingly.

7. After including a one line space between each protocol step, highlight up to 3 pages of protocol text for inclusion in the protocol section of the video. This will clarify what needs to be filmed.

Comment #13: The highlighted text is meant to be filmed.

8. Please sort the Materials Table alphabetically by the name of the material.

Comment #14: The requested changes have been performed.

Reviewers' comments:

Reviewer #1:

Lyhne et al describe their protocol for bi-ventricular PV loop in pigs. The review is well-detailed and explains some important troubleshooting steps. The protocol outlines the steps categorically and appears largely reproducible with exception to variation in animal handling/housing protocols, type of anesthesia applied, availability of exact sheaths, and PV loop recording program. This article should be understandable to experienced users of PV loop applications. For inexperienced and/or junior scientists, the protocol is still straightforward but could clarify some details to aid understanding of the procedure.

We thank the Reviewer for his/her kind comments.

Below are more specific comments.

Section 2 It may be worthwhile to mention preparation of dilators with sheath insertion and to suggest ideal views with fluoroscopy.

Comment #15: We thank the Reviewer for this suggestion. The following has been added to section 2.10 and 3.5, respectively.

"*Consider using a dilator in a two-step approach for the larger sheaths*." And

"*Anterior-posterior view of fluoroscopy is sufficient for all described procedures*."

Section 4 For those lacking experience in right heart catherization, it may be beneficial to provide an explanation for positioning the 7F sheath through the left external jugular vein into the IVC and the subsequent use of a 16F sheath.

Comment #16: Placement of the 7F sheath is described in Section 2.7. Section 4.4-4.6 describes the exchange of the 7F sheath to a 16F by the Seldinger technique. To clarify this, section 4.5 has been edited to:

"*Extract the 7F sheath leaving the guidewire in the venous circulation. Compress on the entry point of the skin to avoid bleeding. Using the Seldinger technique, removed the 7F sheath from the guidewire and exchange it with the 16F Check-Flo sheath*"

Section 4.15 &5 5.10 - in our experience, floating catheter rather causes more extra-beats. It should be fixed without compressing the endocardium too much.

Comment #17: We thank the Review for sharing his/her experience. Interestingly, we have different experiences in this matter. To help the reader of the manuscript troubleshooting, we will provide both information. The following has been added to section 4.15 and 5.10:

"*Occasionally, floating catheter may cause extra-beats. If so, try fixating it without compressing the endocardium too much*."

Section 7.5 Catheter-cross talk is the unique problem to bi-ventricular PV loop. Examples of crosstalk and how changing the frequency (setting description) can improve the data would be an important point for this protocol.

Comment #18: We certainly agree that electrical crosstalk will make biventricular PV recordings impossible. As stated in section 7.5, our PV boxes are manufactured with two different recording frequencies which eliminates this problem.

Section 8.2 Please spell out APL

Comment #19: The correction has been made.

Section 8.6 Examples of simultaneous occlusion loops would be informative (both loops and time-P & time-V waves)

Comment #20: We thank the Reviewer for this suggestion. As per JoVE instructions, such informative examples are referenced in Representative Results rather than during the protocol. The following has been added to section 8.6:

"*See Representative Results for exemplification."*

And to Representative Results:

"*The serial connection of the two ventricles cause a timewise shift in preload reduction (See section 8.6). RV preload is quickly reduced by the IVC balloon, but LV preload is not reduced until RV output has decreased by its lack of preload, see Figure 7A. Gradual reduction in preload will*

cause a family of loops with gradual reduction in volume and pressure to both the LV and RV (Figure 7B-C).”

The following Figure Legend has been added:

***“Figure 7: Preload reduction by inferior vena cava balloon inflation**. (A) shows simultaneous recordings of pressure, volume, phase and magnitude from the left ventricle (top) and the right ventricle (bottom). Please note, how pressure and volume is reduced in the right ventricle prior to the reduction in the left ventricular pressure and volume. Accordingly, the inferior vena cava balloon must be inflated long enough to cause preload reduction in both ventricles (section 8.4-8.6). (B) and (C) shows representative family of loops during such preload reduction for the left ventricle (B) and the right ventricle (C).“*

Section 8.9 Use of average on non-linear data is not recommended. It is probably better to take the relationship with highest r (fit).

Comment #21: We appreciate the Reviewer’s insightful comment. For the end-systolic elastance (Ees) and the preload-recruitable stroke work, a linear fit is most often used. For diastolic function, the end-diastolic elastance (Eed) is often discussed in the literature if it should be linear or exponential. Such discussion is beyond the scope of this paper, the same for choice of statistical analysis. We have edited section 8.9 which now states:

“Consider to do three satisfactory occlusions for the statistical analyses to increase robustness.”

Reviewer #2:

Manuscript Summary:

The authors are presenting manuscript on the methodology of simultaneous pressure-volume catheter insertion to both right and left ventricles and the recording with the commercially available device. The manuscript is well written, the instructions are easy to understand, and figures are instructive.

We thank the Reviewer for noticing pedagogical aspects which were our focus.

Nevertheless, the Introduction and Discussion sections are rather short, commenting only on the described technique, but not putting it into the context of general applications. A detailed description of the procedure, but without any innovation when compared to previously published material. There are no measured results provided. I would expect to see at least a comparison with other available methods for estimation of heart function. Overall, the manuscript reminds more of a user instructions manual then of a research paper - all presented methods are already well known, previously described, and used in animal experiments in large animal models.

Major Concerns:

- For the demonstration of the method, it would be nice to present your own representative results and relate them to other heart assessment (ECHO? Ventriculography? Thermodilution?)

Comment #22: The Reviewer emphasizes an important aspect of validation. However, admittance PV measurements has been validated against both MRI and echocardiography (doi:

10.1113/expphysiol.2013.074179, doi: 10.1113/expphysiol.2012.070821, and doi: 10.14814/phy2.287). Due to the sensitive nature of these PV catheters where other devices would cause unwanted noise, and due to PV catheters' superiority of hemodynamic evaluation, we did not use other heart assessments in our studies.

- Would it be possible to compare the cardiac output of the RV and LV from your acquired data?

Comment #23: We thank the Reviewer for this suggestion. The following has been added to the Results section:

"To compare our simultaneous PV recordings in the RV and LV, we performed a linear regression of the bi-ventricular CO measurements from our largest study[18] with the highest number of simultaneous measurements (n=379). We found, that the slope was 1.03 (95%CI 0.90-1.15) with a Y-intercept of 695 (95%CI -2-1392) and r^2=0.40."

- The title says model of acute right ventricular dysfunction - but none is discussed in the article

Comment #24: The Reviewer raises a good point. The title included RV dysfunction as our experience of closed chest, bi-ventricular PV loop recordings stem from such model, but it is the method of evaluation rather than the model that is the aim of the present paper. If acceptable to JoVE, we suggest to change the title to:

"*Closed chest biventricular pressure-volume loop recordings with admittance catheters in a porcine model*"

- There already exists at least one video tutorial on Transonic PV loop from both ventricles https://www.youtube.com/watch?v=EdyB9Yrha7k
Can you specify the advantage of your manuscript over it?

Comment #25: We appreciate the Reviewer's reference to this video. The Transonic tutorial is very informative and contains lots of background information on the Transonic system, calibration, blood resistivity measurement etc. The tutorial is definitely useful for new users of admittance technology. However, the Transonic tutorial contains few (and sub-optimal) fluoroscopic pictures, only still-images, no instructions in sheaths insertions, no instructions in actual advancement of the catheters into the ventricles (which is definitely the difficult part of experimental research).

Our manuscript is not meant exclusively for admittance-based catheters, as the techniques can be applied for other catheters as well as for other models than RV dysfunction. Contrary to the tutorial, our paper describes the actual instrumentation and includes several troubleshooting suggestions for researchers to use if struggling in the animal lab. We believe, our illustrations are superior, and in combination with the professional filming by JoVE, the actual maneuverers will be shown. Accordingly, we believe that our paper, combined with the later recorded filming, has several advantages over this tutorial, justifying its necessity.

Minor Concerns:

51 - LV/RV interdependence - please explain in better way

Comment #26: That part of the Introduction has been changed and now states:

"…However, *the right and left ventricles exert systolic and diastolic interdependence* due to their serial and parallel connections *within the tight pericardium*[11]. *Changes in output or size of one ventricle will affect size, loading conditions or perfusion of the other ventricle….*"

92 - add reference to hypothermia-induced proarrhythmogenicity or revise

Comment #27: A reference (doi:10.1093/cvr/cvy305) has been added and the reference list updated accordingly.

100 - intra-device thrombosis - better intra-luminal

Comment #28: We agree, the correction has been made.

177 - sheath into thoracic cavity.. use better words

Comment #29: In section 4.6, it now states:

"… until the tip of the sheath (not the dilator) *has reached the level of the superior vena cava…"*

195, 224 - without the catheter tip touching the endocardium, how do you obtain sufficient stability?

Comment #30: We thank the Reviewer for identifying this lack of information. The following has been added to section 4.15 and 5.10:

"Fixate the PV catheter to the external end of the sheath to ensure stability of catheter positioning."

223 - "On inside"

Comment #31: The error has been corrected to "Once inside".

234 - is using air recommended?

Comment #32: We know of others who use air in their balloon. "Air," has been deleted from the revised manuscript.

277 - "is" -> "if"?

Comment #33: Correct, we thank the Reviewer for identifying this mistake. The correction has been made.

Reviewer #3:

Manuscript Summary:

The Athours describe a well known closed chest porcine model for invasive assasment of cardiac function. The new thing here is that they simultaneosly place PV catheters in the right and left ventricles.

We thank the Reviewer for his/her time.

Major Concerns:

The shape of the right ventricle is not optimal for assessing conductace volumetry by a straight catheter. Have the Authrours tried to place two catheters in simultaneoslyin the right ventricle

(retrograde and antagrade?) If so, is the shape and phases of the PV loop similar with both aproaches?

Comment #34: The Reviewer raises an important question. The right ventricle is, indeed, troublesome regardless method of evaluation due to its geometry. We did not try the retrograde approach to the right ventricle as it would require opening of the thoracic cavity and thereby cause significant physiological changes. Those are avoided in our closed chest approach. The following as been added to the limitations section of the paper:

"Thirdly, the shape of the RV is not optimal for assessing volumetry by a straight catheter, and minor parts of the RV outflow tract might be missed with our antegrade approach. However, repeated measurements before/after interventions with a fixated catheter will limit this bias, and PV loop recordings in general offer a number of hemodynamic variables outweighing this concern."