

Video Article

# Invasive Hemodynamic Monitoring of Aortic and Pulmonary Artery Hemodynamics in a Large Animal Model of ARDS

Rahel Kluttig<sup>1</sup>, Till Friedheim<sup>1</sup>, Christoph Behem<sup>1</sup>, Niko Zach<sup>1</sup>, Rosannis Brown<sup>1</sup>, Michael Graessler<sup>1</sup>, Daniel Reuter<sup>1</sup>, Christian Zöllner<sup>1</sup>, Constantin Trepte<sup>1</sup>

<sup>1</sup>Universitätsklinikum Hamburg-Eppendorf

Correspondence to: Rahel Kluttig at [R.kluttig@uke.de](mailto:R.kluttig@uke.de)

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## Abstract

One of the leading causes of morbidity and mortality in patients with heart failure is right ventricular (RV) dysfunction, especially if it is due to pulmonary hypertension. For a better understanding and treatment of this disease, precise hemodynamic monitoring of left and right ventricular parameters is important. For this reason, it is essential to establish experimental pig models of cardiac hemodynamics and measurements for research purpose.

This article shows the induction of ARDS by using oleic acid (OA) and consequent right ventricular dysfunction, as well as the instrumentation of the pigs and the data acquisition process that is needed to assess hemodynamic parameters. To achieve right ventricular dysfunction, we used oleic acid (OA) to cause ARDS and accompanied this with pulmonary artery hypertension (PAH). With this model of PAH and consecutive right ventricular dysfunction, many hemodynamic parameters can be measured, and right ventricular volume load can be detected.

All vital parameters, including respiratory rate (RR), heart rate (HR) and body temperature were recorded throughout the whole experiment. Hemodynamic parameters including femoral artery pressure (FAP), aortic pressure (AP), right ventricular pressure (peak systolic, end systolic and end diastolic right ventricular pressure), central venous pressure (CVP), pulmonary artery pressure (PAP) and left arterial pressure (LAP) were measured as well as perfusion parameters including ascending aortic flow (AAF) and pulmonary artery flow (PAF). Hemodynamic measurements were performed using transcardiopulmonary thermodilution to provide cardiac output (CO). Furthermore, the PiCCO2 system (Pulse Contour Cardiac Output System 2) was used to receive parameters such as stroke volume variance (SVV), pulse pressure variance (PPV), as well as extravascular lung water (EVLW) and global end-diastolic volume (GEDV). Our monitoring procedure is suitable for detecting right ventricular dysfunction and monitoring hemodynamic findings before and after volume administration.

## Video Link

The video component of this article can be found at <https://www.jove.com/video/57405/>

## Introduction

Right ventricular (RV) dysfunction is a major cause of morbidity and mortality in patients with heart failure<sup>1</sup>, especially if the underlying cause is pulmonary hypertension<sup>2</sup>. The RV pumps blood into the low-resistance pulmonary system, which is normally associated with high compliance. Therefore, the RV is characterized by low peak systolic pressure. It also generates one sixth the stroke work compared with the left ventricle (LV)<sup>3</sup>. Due to its thinner muscle, the RV is very vulnerable to a change in pre- and afterload<sup>4,5</sup>. The isovolumic phases of contraction and relaxation during systole and diastole in the RV are not as distinct as in the LV. The examination of left and right ventricular hemodynamic parameters is highly important in therapy of critically ill patients with acute right heart distress<sup>4,7</sup>, because RV failure increases short-term mortality significantly<sup>6</sup>.

Preload parameters like the central venous pressure (CVP) and left ventricular preload parameters like pulmonary capillary wedge pressure (PCWP) have been used for a long time to determine volume status of patients. Lately, it has been shown that these parameters alone are not suitable to detect a patient's need of fluids<sup>8,9,10</sup>. Recognizing fluid responsiveness is essential to detect and treat volume deprivation and volume overload in patients with RV dysfunction. Avoiding volume overload is essential to decrease the mortality and length of intensive care unit (ICU) stay in these patients.

With this study, we established a pig model of right ventricular dysfunction that is consistent and replicable. Due to its similarity to humans, it is necessary to establish consistent and reproducible experimental large animal models of cardiac hemodynamics and measurements for research purpose.

## Protocol

This prospective experimental trial with 21 anesthetized male and female domestic pigs (German landrace) at the age of 3–6 months with a body weight between 45–55 kg was approved by the Governmental Commission on the Care and Use of Animals of the City of Hamburg (Reference-No. 18/17). According to the ARRIVE guidelines, all experiments were carried out and all animals received care in compliance with the 'Guide for the Care and Use of Laboratory Animals' (NIH publication No. 86-23, revised 1996)<sup>11</sup>.

### 1. Flow Probe Two-point Calibration

1. Put flow probes in deionized water and connect the probe to the transonic flow probe system by putting the plug into the perivascular flow module.
2. Open the data analysis software (e.g., LabChart 8).
3. For a two-point calibration, start a measurement by setting the flow probe system to **Zero** and after a few seconds to **Scale**.
4. In the data analysis software window, go to **Units Conversion** and choose two-point calibration. Mark a baseline to set to zero. Then, mark an area with 10 L/min and set to 1 V as a preset value.
5. Repeat the procedure for the other probe.

### 2. Millar Catheter Calibration

1. Prior to the zeroing and calibration, pre-soak the tip of the catheter in sterile body temperature warm water for 30 min.
2. Connect the Millar catheter to the bridge amplifier by putting the plug into the bridge amplifier module.
3. Start the data analysis software.
4. Put the tip of the catheter into the pneumatic zeroing tool, set the value to 0 mmHg and start a measurement by clicking **Start** in the program.
5. Keep the measurement running and set the pneumatic zeroing tool to 100 mmHg. Stop the measurement by clicking **Stop**.
6. Run the data analysis software by pressing **Start** and then press **Stop**. Click **Amplify** in the window of bridge and choose **Units**. Set the baseline of 0 and 100 mmHg, accordingly. According to the preset value for calibration that the software provides, the catheter is now calibrated for all body pressures.
7. Repeat the procedure for the other Millar catheter.

### 3. Preparation of the Pig

1. Medicate the pig by injecting 20 mg/kg of Ketanest, 4 mg/kg of Azaperon and 0.1 mg/kg Midazolam intramuscularly and place a 20 G IV-line into a vein of the ear.
2. Place ECG stickers on the chest and oxygen probe on the tail.
3. Administer pure oxygen (15–18 L/min) via the pig's nose using a mask and surgically prepare down to the trachea.
4. Put a loop around the trachea, use a scalpel (11 blade) to make an incision into the trachea and place an 8.5 Mallinckrodt tube into it for a safe airway. Fix the tube with the preset loop and close the skin with sutures.
5. Begin anesthesia with Sevoflurane using a MAC of 0.9 (in adolescent pigs equivalent to an endexpiratory concentration of 2.0%) and infusion of 0.01 mg/(kg·h) Fentanyl. Start mechanical ventilation with a tidal volume of 10 mL/kg, a rate of 14/min, and a positive end expiratory pressure (PEEP) of 7 mmHg. Set the inspiratory oxygen rate (FiO<sub>2</sub>) to 0.3. After 10 min depth of anesthesia is deep enough to perform surgery safely. No elevation of HR and BP should be detected.
6. Maintain fluid balance at basal volume rate of 10 mL/(kg·h) cristalloid using an infusion pump.
7. Gently clean the pig's skin using soap water. Use a skin disinfection solution containing povidone-iodine to decrease skin contamination.

### 4. Vital Parameter Measurements

1. Use an ultrasound for inserting a 5 F thermistor tipped arterial catheter into the right femoral artery, an 8 F introducer sheath into the left femoral artery, a central venous catheter and an 8 F introducer sheath into the jugular vein (**Figure 1**).
2. Place the catheter placement using Seldinger's technique<sup>12</sup>.
  1. Place a needle into the target vessel under ultrasound vision.
  2. Put a wire through the needle into the vessel, verify the correct placement of the wire using ultrasound and keep the wire in the vessel throughout the whole procedure. Remove the needle and place a dilator onto the wire.
  3. With gentle pressure, put the dilator through the skin into the vessel using the wire as guidance. Remove the dilator, put the catheter onto the wire, make sure to the end of the wire is seen at the end of the catheter and place the catheter it into the vessel.
  4. Remove the wire by gently pulling it out of the catheter.
3. Insert a 7F pulmonary artery catheter (PAC) into the 8F introducer sheath and place it in the RV. If needed for taking mixed venous blood gas samples, insert the PAC further into the PA until a pulmonary artery curve is shown on to the monitor and pull it back after receiving the samples.
4. Insert the first Millar-tip catheter into the 8F introducer sheath in the left femoral artery and placing it in the aorta.
5. Perform a mini-laparotomy (approximately 5–10 cm is enough) above the symphysis by using the electrocautery for prepping down to the linea alba.
  1. Open the linea alba with scissors and pull out the bladder very gently.
  2. Put a purse string suture in the bladder using a 3/0 suture and make an incision into the bladder with a scalpel (11 blade).

3. Insert a urinary catheter into the bladder, inflate the catheter's balloon with water and fix it using the purse-string suture. Close the abdomen with a 3/0 suture.

## 5. Surgical Preparation of the Heart

1. Before opening the chest increasing the  $\text{FiO}_2$  to 1.0 and administer 0.1 mg  $\text{kg}^{-1}$  pancuronium initial bolus intravenously<sup>13</sup>.
2. Perform a median sternotomy.
  1. Use the electrocautery for prepping down to the sternum. Gently dissect the sternum from the surrounding tissue before dividing the bone with an oscillating saw.
  2. Use the electrocautery to reduce bleeding and seal the sternum with bone wax. Place a sternal rib spreader between the two halves of the opened sternum and widely open the chest as much as needed for surgery by twisting the handle on the device.
3. Open the pericardium gently using scissors and forceps and fix it to the skin with a 2/0 suture.
4. Dissect down the pulmonary and the artery ascending aorta very gently to avoid bleeding. Carefully place the ultrasound flow probes around both arteries, respectively (**Figure 2**).
5. Place 2 purse string sutures in the pulmonary artery using a 5/0 suture. Use a scalpel (11 blade) to make a small stitch incision (approximately 1 mm) in the middle of the purse strings and place the Millar catheter into the pulmonary artery before fixing it (**Figure 3**).
6. Carefully clamp the LAA and place 2 purse string sutures in it using a 4/0 suture. Make a small incision and place a central venous line into the left atrium before fixing it using the purse string sutures (**Figure 3**).
7. Close the pericardium by suturing a sterile glove onto it, to maintain hemodynamics reliable (**Figure 4**). Perform the sternal closure with wires and close the skin with a 3/0 suture.

## 6. Assessment and Data Acquisition

1. Start each measurement with 2 min of AO and PA flow measurements, as well as AO and PA pressure measurements using the data analysis software by clicking **Start** and **Stop** button in the program.
2. Perform transcadiopulmonary thermodilution to provide cardiac output (CO) as well as pulse pressure variance (PPV) and stroke volume variance (SVV) by using the PiCCO2 system. To start the measurement, click the **TD | Start**.
3. Consecutive inject 15 mL of 10 °C cold saline into a thermistor at the central venous line in the jugular vein three times for thermodilution at each measurement step.
4. Take an arterial, central venous and mixed venous blood gas sample after each transcadiopulmonary thermodilution measurement step.

## 7. Volume Optimizing

1. After a baseline measurement M0 (steps 6.1–6.4) of all parameters, administer a volume loading step using 5 mL/kg of colloidal infusion (Voluven) using an infusion pump that is connected to the central venous line.
2. After 5 min of equilibration, start another measurement step M1 (steps 6.1–6.4). If the newly generated cardiac output measured by thermodilution using the PiCCO2 system (see step 6.2–6.3) does not increase compared to the formerly measured CO by at least 10%, start another volume loading step (step 7.1).
3. Continue with volume loading and equilibration steps until there is no more increase in CO of more than 10%. Now, a balanced fluid status is reached.

## 8. Induction of ARDS with Right Ventricular Dysfunction

1. Increase the  $\text{FiO}_2$  to at least 0.5 to 0.8 as required to maintain a  $\text{SpO}_2$  of at least 90%.
2. Induce an ARDS with consecutive right ventricular dysfunction by infusion of oleic acid (OA) (0.03–0.06 mL/kg for about 2 h).
3. Use continuous administration of adrenalin using a perfusor (3 mg of adrenalin in 50 mL of saline) to keep hemodynamics stable. Increase the infusion rate as required to maintain a mean arterial pressure of 50 mmHg.
4. Add calcium, magnesium and antiarrhythmics (1% Lidocain) as required during the infusion of OA to maintain a stable sinus rhythm.

## 9. Volume Optimizing

1. After induction of mild to moderate ARDS, perform another measurement of all parameters (M2) by completing steps 6.1–6.4.  
NOTE: Now, the baseline model for hemodynamic measurements in ARDS in a pig model is set. For further investigation on volume responsiveness in ARDS and right ventricular dysfunction start to reduce volume load by taking as much blood as need per protocol or increasing volume load by adding a defined amount of infusion.

## 10. Finalization

1. After finishing the measurements euthanize the pigs while under anesthesia by injecting 1mmol/kg potassium chloride intravenously.

## Representative Results

Our animal model shows a broad variety of hemodynamic parameters in pigs. Due to its similarity in size and hemodynamics, one can easily use the exact same equipment as used in humans to get similar results. However, anesthesia values are based on experience and may change based on weight/ age/ strain of pig. A veterinarian should be consulted to evaluate anesthetic plan.

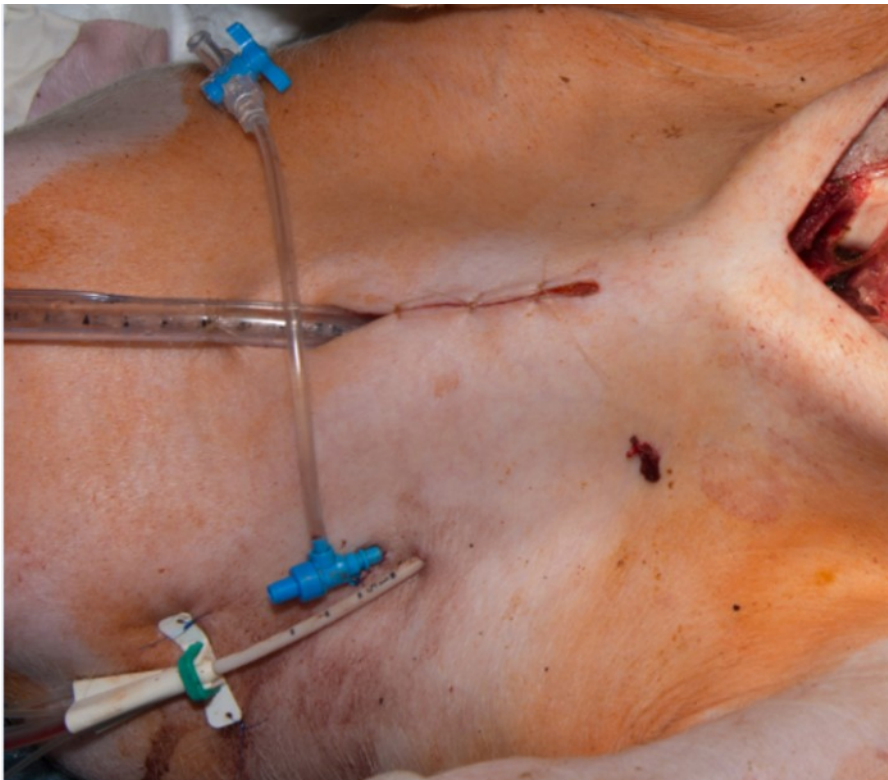
Results of previous OA induced acute lung injury (ALI) models were inconsistent<sup>13,14,15,16</sup>. Former protocols stated that OA has been administered mixing it with blood, normal saline, or purely administering it into the heart, a central vein or a peripheral vein in doses of 0.6–2 mL/kg bodyweight<sup>17,18</sup>. We tried all of the above methods and found out that purely administering low doses of OA (0.03–0.06 mL/kg for about 2 h) achieved the most consistent results of ARDS without losing any animals due to respiratory failure or severe acute right heart failure.

First, we were able to show that the intravenous infusion of OA is an easy and good model to induce ARDS as shown before. Depending on the amount of OA administered, one gets a mild to severe lung injury up to death<sup>13</sup>. It has been shown that an amount of about 0.1 mL/kg OA is mostly used to have a moderate ALI<sup>16,18</sup>.

To get a mild to moderate ARDS that can be used for further investigation, it is sufficient to inject 0.03–0.06 mL/kg OA. After the administration of this small amount of OA, the oxygenation index decreased from  $516.83 \pm 50.25$  mmHg to  $181.19 \pm 32.25$  mmHg ( $p = 0.0006$ ) (**Figure 6**). The decrease of oxygenated blood is accompanied with a statistically significant increase in carboxylated blood from  $36.71 \pm 4.51$  mmHg to  $46.50 \pm 6.87$  mmHg ( $p = 0.008$ ) (**Figure 7**).

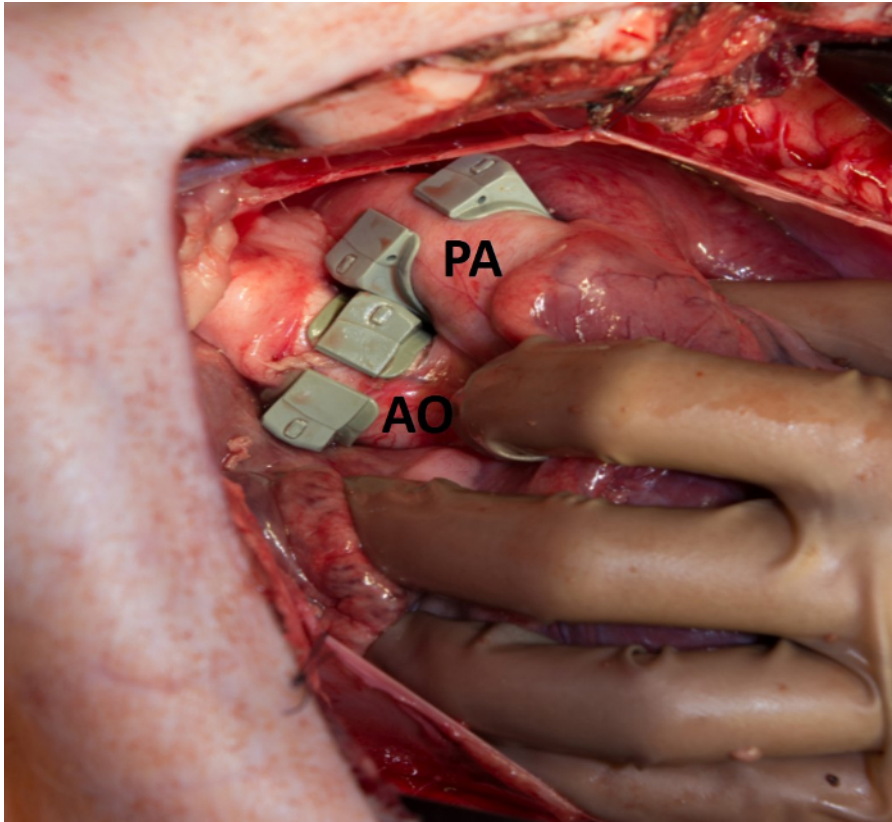
Pulmonary hypertension is defined as a PAP of more than 25 mmHg, a PCWP (which equals the left arterial pressure) of  $\leq 15$  mmHg and a pulmonary vascular resistance (PVR)  $> 240 \text{ dyn} \times \text{s} \times \text{cm}^{-5}$ <sup>19,20,21</sup>. There is a prevalence of about 1%<sup>17</sup> with this common disease worldwide. The PCWP accurately reflects both normal and elevated LAP and vice versa<sup>18</sup>. In our open-heart animal model, we used a catheter placed in the left atrial to measure this value, because a PAC placed in the pulmonary artery through the pulmonary flow probe could cause incorrect flow measurements (**Figure 5**).

For a correct and especially consistent measurement of the PAP, we used a Millar catheter, which is put directly in the PA and placed in the main pulmonary artery (MPA) about 2 cm after the pulmonary valve.

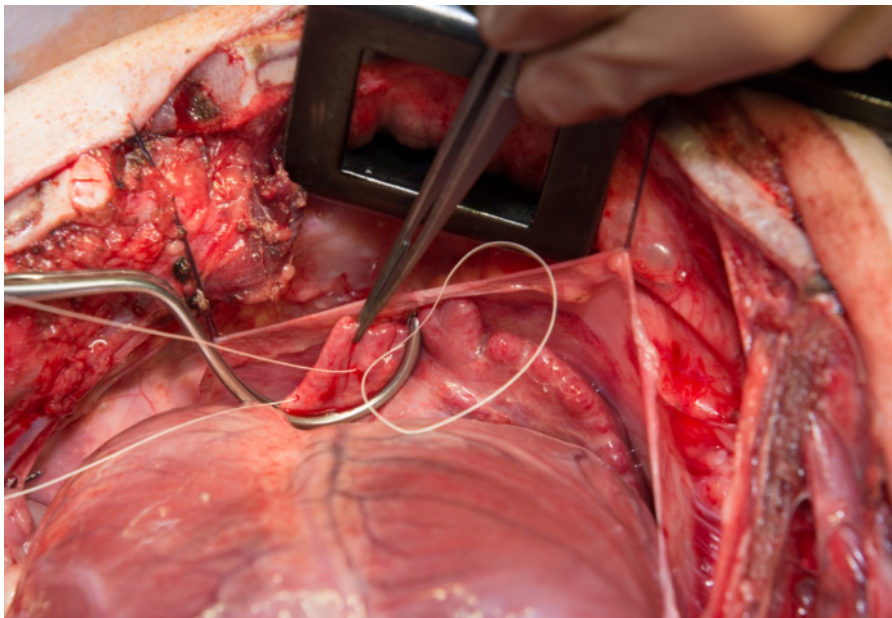


**Figure 1:** For safe and easy airway management during the whole surgery, perform a tracheotomy and placement of an 8.5 tube directly in the trachea. The bigger the inner diameter of the tube, the better for mechanical ventilation during ARDS. The catheters in the right jugular vein and both femoral arteries are placed by ultrasound using Seldinger's technique. [Please click here to view a larger version of this figure.](#)

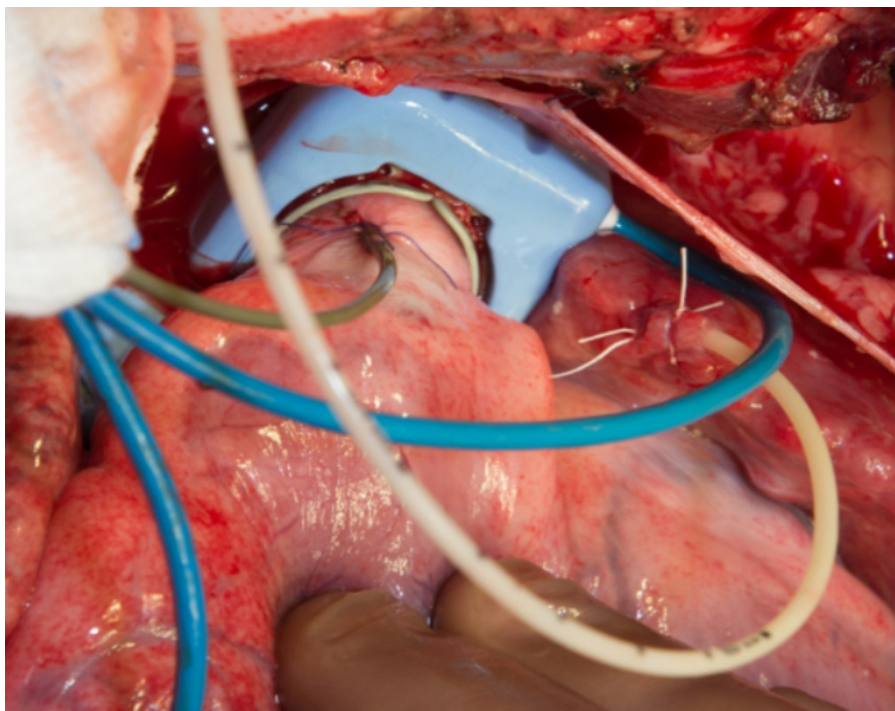




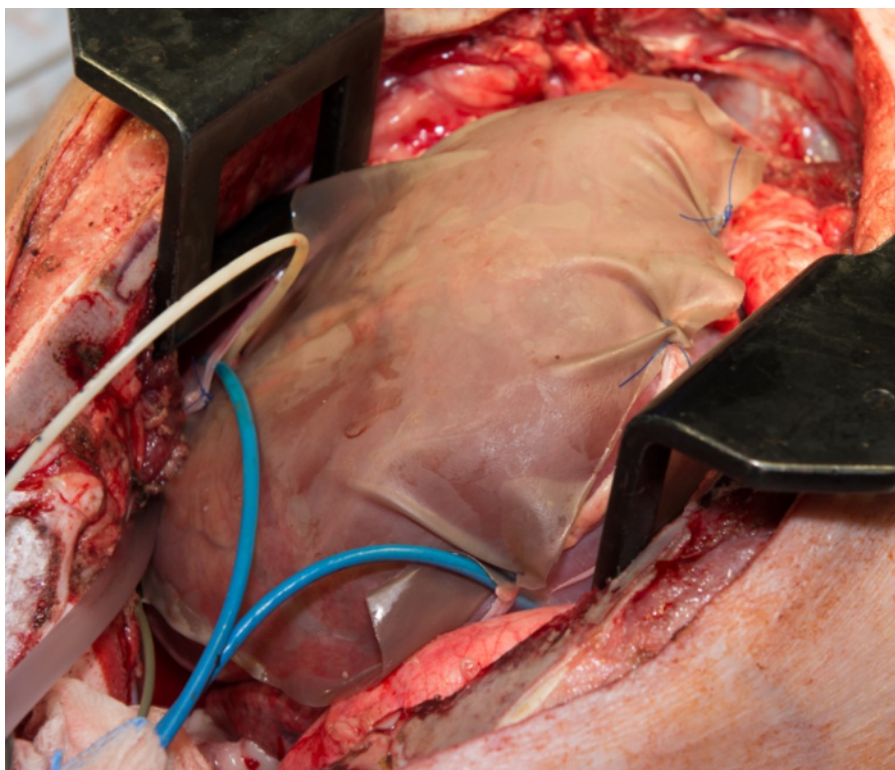
**Figure 2: After opening the pericardium, push the RV and RAA away gently for better visualization of the aorta and pulmonary artery.** Hemodynamics must be monitored closely during these steps due to a decreased cardiac output. Dissect the connective tissue of the cardiac skeleton between the PA and the aorta gently, especially as the PA is very predisposed towards bleeding due to its thinner wall. Choose the right sized chronic lined low probes (mostly 18–20 mm) to put around the aorta and the pulmonary artery. [Please click here to view a larger version of this figure.](#)



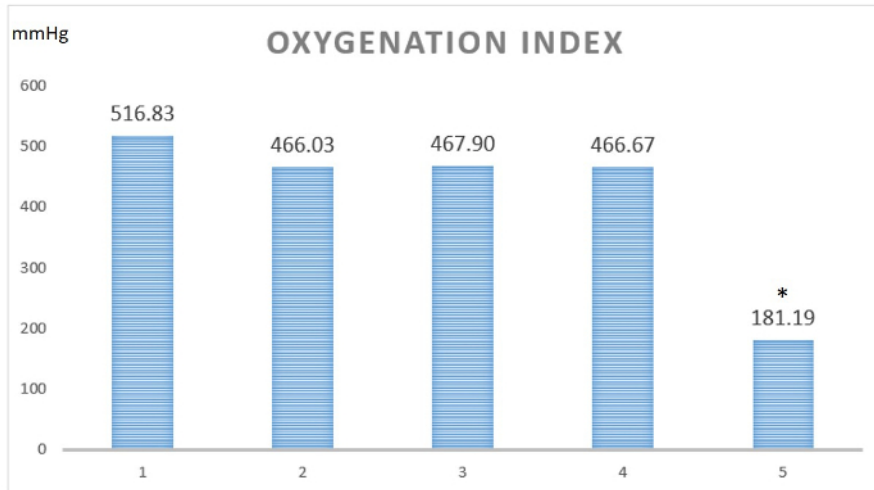
**Figure 3: Use a vascular clamp to fix the LAA and to avoid bleeding.** For safe and secure surgery, place two purse string sutures around an edge of the LAA, making a small incision and putting the catheter into the heart. Quickly open the clamp to position the catheter approximately 5 cm deep into the left atrium while monitoring the pressure curve. Reposition the catheter as needed. Fix the catheter by using the purse string sutures. [Please click here to view a larger version of this figure.](#)



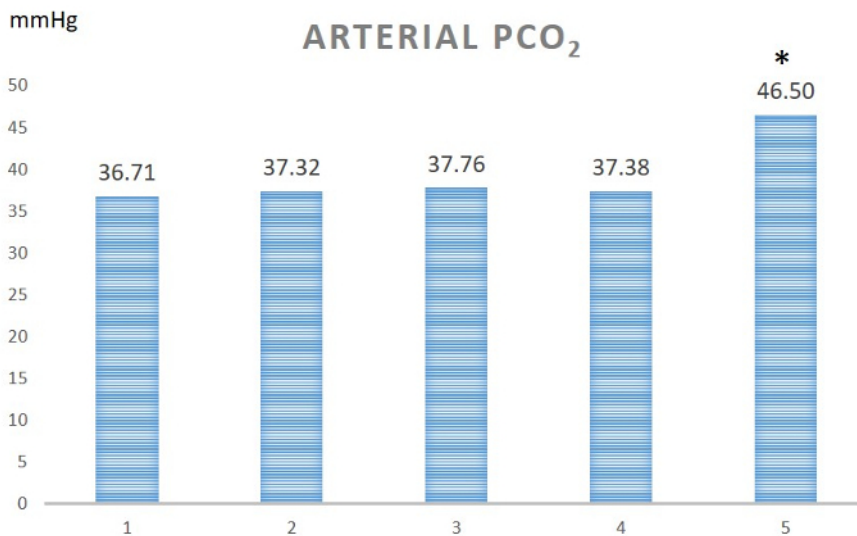
**Figure 4: Very gently put two purse string sutures into the PA.** To avoid unnecessary bleeding, use a tourniquet on one of the purse strings. Make a small incision and put the Millar catheter into the pulmonary artery and immediately pull down the tourniquet. Fix it using both sutures. Impose the probe shell on both aortal and pulmonary flow probes. [Please click here to view a larger version of this figure.](#)



**Figure 5: Use a patch to close the pericardium.** Because opening of the pericardium during cardiac surgery goes along with an increase in CO and stroke work index, we chose to close the pericardium using a patch to maintain hemodynamic conditions similar to the ones prior to surgery<sup>19</sup>. [Please click here to view a larger version of this figure.](#)



**Figure 6:** Since we increased the oxygen fraction due to the pulmonary impairment of the ARDS, the oxygenation index was calculated for each measurement step. We were able to see a decrease from  $516.83 \pm 50.25$  mmHg at the baseline measurement (1) to  $181.19 \pm 32.25$  mmHg ( $p = 0.0006$ ) after administration of OA (5). [Please click here to view a larger version of this figure.](#)



**Figure 7:** Along with the decrease of oxygenated arterial blood goes a statistically significant increase in carboxylated blood after induction of ARDS. The baseline measurement was at  $36.71 \pm 4.51$  mmHg and increased to  $46.50 \pm 6.87$  mmHg ( $p = 0.008$ ) after administration of OA. [Please click here to view a larger version of this figure.](#)

## Discussion

ARDS, complicated by pulmonary hypertension, is a very deadly disease. For patients suffering from this condition, further information about treating it is necessary. When working and researching with living creatures, it is very important to be as sensible as possible. In this case it is necessary to gather as much information as possible in one experiment.

There are some critical surgical steps in an open-beating heart model like this. To not use pigs unnecessarily, there must be an experienced surgeon to dissect the heart skeleton between the ascending aorta and the pulmonary artery while hemodynamics are unstable due to the pressure on the RV and RA. Another critical step is putting the Millar tip catheter into the pulmonary artery. To get a better exposure of the surgical field, the right ventricular outflow tract (RVOT) needs to be pushed away very gently. With the right amount of pressure, it is possible to have good visibility and stability of the PA. This makes it easier to take small bites with the 5.0 suture and decreases the risk of PA bleeding or injury.

When measuring hemodynamics, losing a great amount of blood and thus changing the hematocrit significantly can influence the measurements and the results<sup>20</sup>. When placing the catheter into the artery, using a tourniquet first and making a very small incision to fix the catheter quickly could prevent any blood loss. Ensure that all small bleedings are stopped before insertion of the Millar catheter, because electrocautery can damage the catheter (as described in the catheters manual). After closing of the pericardium and the sternum small bleedings can accumulate



over time and cause changes in hematocrit or cause a pericardial tamponade with significant changes in hemodynamics. This could cause a termination of the experiment.

When cutting into the LA, one must be careful. The LA is the pacemaker of the heart and it can react with heart rhythm disturbances when touching it with cold metal instruments. Before putting the clamp gently around the LAA, administration of magnesium could prevent atrial fibrillation (AF). Rhythm disturbances like AF have great impact on left as well as right ventricular hemodynamics<sup>21</sup>.

## Disclosures

Daniel A. Reuter is a member of Pulsion Medical Advisory Board. Constantin J.C. Trepte has received honorary award for lectures by Maquet. All other authors declare no conflicts of interest.

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