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TITLE:

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

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KEYWORDS:

Hemodynamic monitoring; right ventricular dysfunction; ARDS; right ventricular parameters; pulmonary artery hypertension; Millar catheters; flow probe

SUMMARY:

We present a protocol of creating right ventricular dysfunction in a pig model by inducing ARDS. We demonstrate invasive monitoring of left and right ventricular cardiac output using flow probes around the aorta and the pulmonary artery, as well as blood pressure measurements in the aorta and pulmonary artery.

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.

INTRODUCTION:

Right ventricular (RV) dysfunction is a major cause of morbidity and mortality in patients with heart failure[1], especially if the underlying cause is pulmonary hypertension[2]. 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)[3]. Due to its thinner muscle, the RV is very vulnerable to a change in pre- and afterload[4,5]. 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[4,7], because RV failure increases short-term mortality significantly[6].

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[8-10]. 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)[11].

1. Flow Probe Two-Point Calibration

1.1. 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.

1.2. Open the data analysis software (*e.g.*, LabChart 8).

1.3. For a two-point calibration, start a measurement by setting the flow probe system to **Zero** and after a few seconds to **Scale**.

1.4. 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.

1.5. Repeat the procedure for the other probe.

2. Millar Catheter Calibration

2.1. Prior to the zeroing and calibration, pre-soak the tip of the catheter in sterile body temperature warm water for 30 min.

2.2. Connect the Millar catheter to the bridge amplifier by putting the plug into the bridge amplifier module.

2.3. Start the data analysis software.

2.4. 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.

2.5. Keep the measurement running and set the pneumatic zeroing tool to 100 mmHg. Stop the measurement by clicking **Stop**.

2.6. 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.

2.7. Repeat the procedure for the other Millar catheter.

3. Preparation of the Pig

3.1. 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 20G IV-line into a vein of the ear.

3.2. Place ECG stickers on the chest and oxygen probe on the tail.

3.3. Administer pure oxygen (15-18 L/min) via the pig's nose using a mask and surgically prepare down to the trachea.

3.4. 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.

3.5. Begin anesthesia with Sevoflurane using an end expiratory 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_2) to 0.3. After 10 minutes depth of anesthesia is deep enough to perform surgery safely. No elevation of HR and BP should be detected.

3.6. Maintain fluid balance at basal volume rate of 10 mL/(kg·h) cristalloid using an infusion pump.

3.7. 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

4.1. 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**).

4.2. Place the catheter placement using Seldinger's technique[12].

4.2.1. Place a needle into the target vessel under ultrasound vision.

4.2.2. 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.

4.2.3. 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.

4.2.4. Remove the wire by gently pulling it out of the catheter.

4.3. 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.

4.4. Insert the first Millar-tip catheter into the 8F introducer sheath in the left femoral artery and placing it in the aorta.

4.5. Perform a mini-laparotomy (approximately 5 -10 cm is enough) above the symphysis by using the electrocautery for prepping down to the linea alba.

4.5.1. Open the linea alba with scissors and pull out the bladder very gently.

4.5.2. 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).

4.5.3. 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

5.1. Before opening the chest, increase the fiO_2 to 1.0 and administer 8 mg of pancuronium, intravenously.

5.2. Perform a median sternotomy.

5.2.1. 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.

5.2.2. 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.

5.3. Open the pericardium gently using scissors and forceps and fix it to the skin with a 2/0 suture.

5.4. 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**).

5.5. 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**).

5.6. 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**).

5.7. 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

6.1. 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.

6.2. Perform transcardiopulmonary 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**.

6.3. 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.

6.4. Take an arterial, central venous and mixed venous blood gas sample after each transcardiopulmonary thermodilution measurement step.

7. Volume Optimizing

7.1. 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.

7.2. 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).

7.3. 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

8.1. Increase the fiO_2 to at least 0.5 to 0.8 as required to maintain a spO_2 of at least 90%.

8.2. Induce an ARDS with consecutive right ventricular dysfunction by infusion of oleic acid (OA) (0.03-0.06 mL/kg for about 2 h).

8.3. 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.

8.4. Add calcium, magnesium and antiarrhythmics (1% Lidocain) as required during the infusion of OA to maintain a stable sinus rhythm.

9. Volume Optimizing

9.1. 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**

10.1. After finishing the measurements, euthanize the pigs were by injecting 40 mmol of 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.

Results of previous OA induced acute lung injury (ALI) models were inconsistent[13-16]. 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[17,18]. 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[13]. It has been shown that an amount of about 0.1 mL/kg OA is mostly used to have a moderate ALI[16,18].

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 ± 50.25 mmHg to 181.19 ± 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 ± 4.51 mmHg to 46.50 ± 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 ≤ 15 mmHg and a pulmonary vascular resistance (PVR) > 240 dyn × s × cm^{-5} [19-21]. There is a prevalence of about 1%[17] with this common disease worldwide. The PCWP accurately reflects both normal and elevated LAP and vice versa[18]. 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.

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.

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.

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.

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 [19].

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 ± 50.25 mmHg at the baseline measurement (**1**) to 181.19 ± 32.25 mmHg ($p = 0.0006$) after administration of OA (**5**).

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 ± 4.51 mmHg and increased to 46.50 ± 6.87 mmHg ($p = 0.008$) after administration of OA.

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 have good visualibility 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[20]. 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[21].

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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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Figure 1

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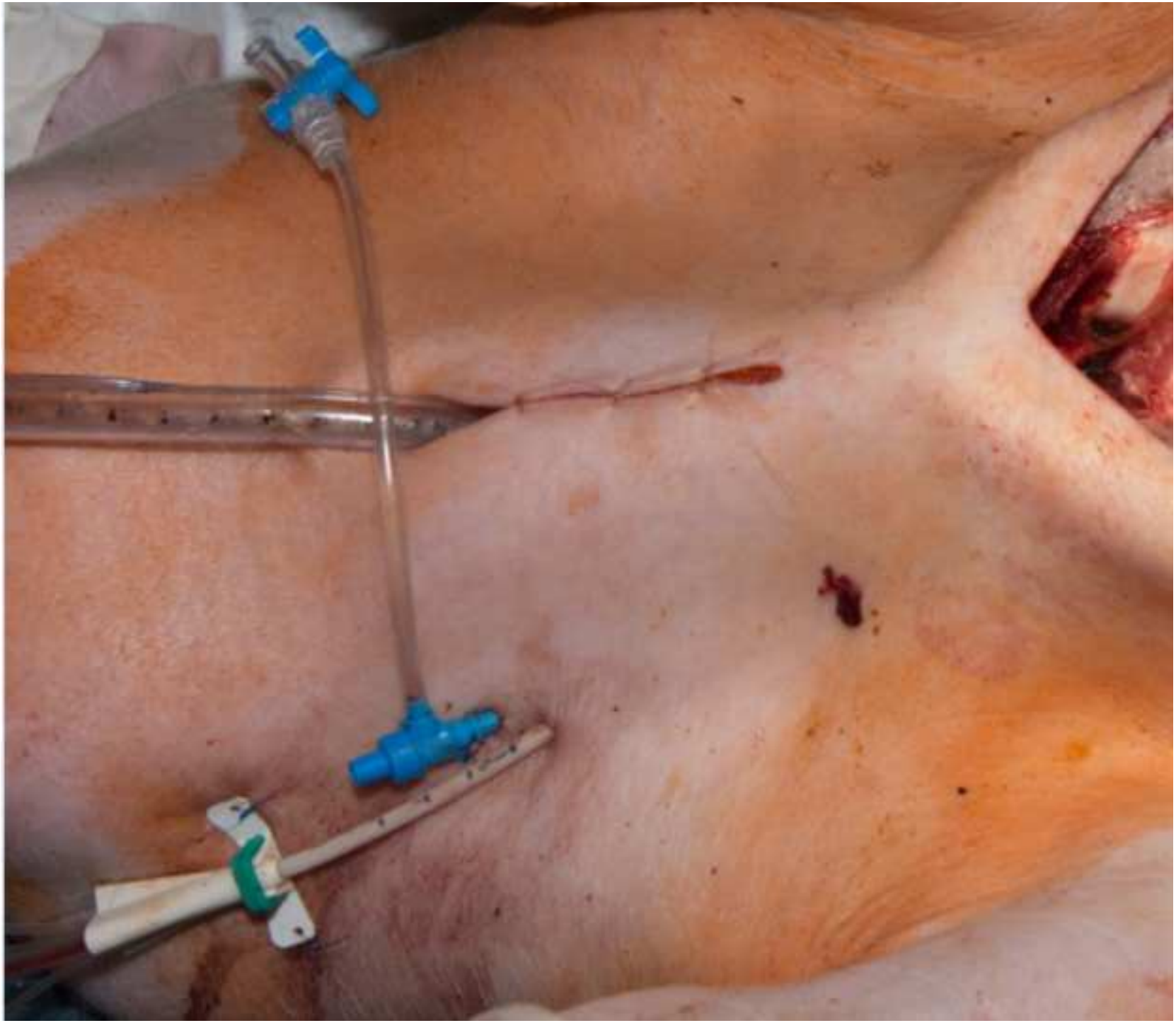

Figure 2

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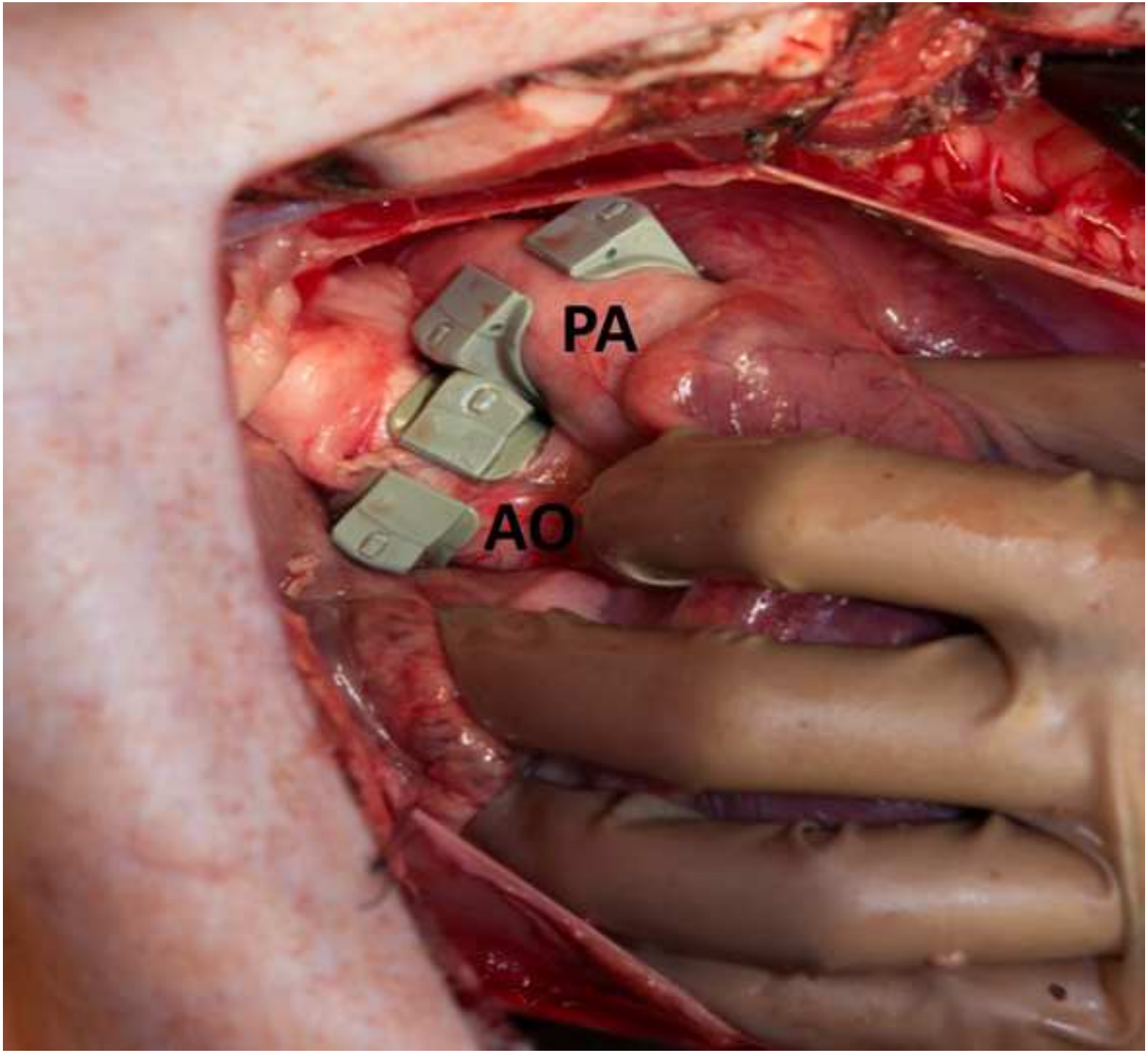

Figure 3

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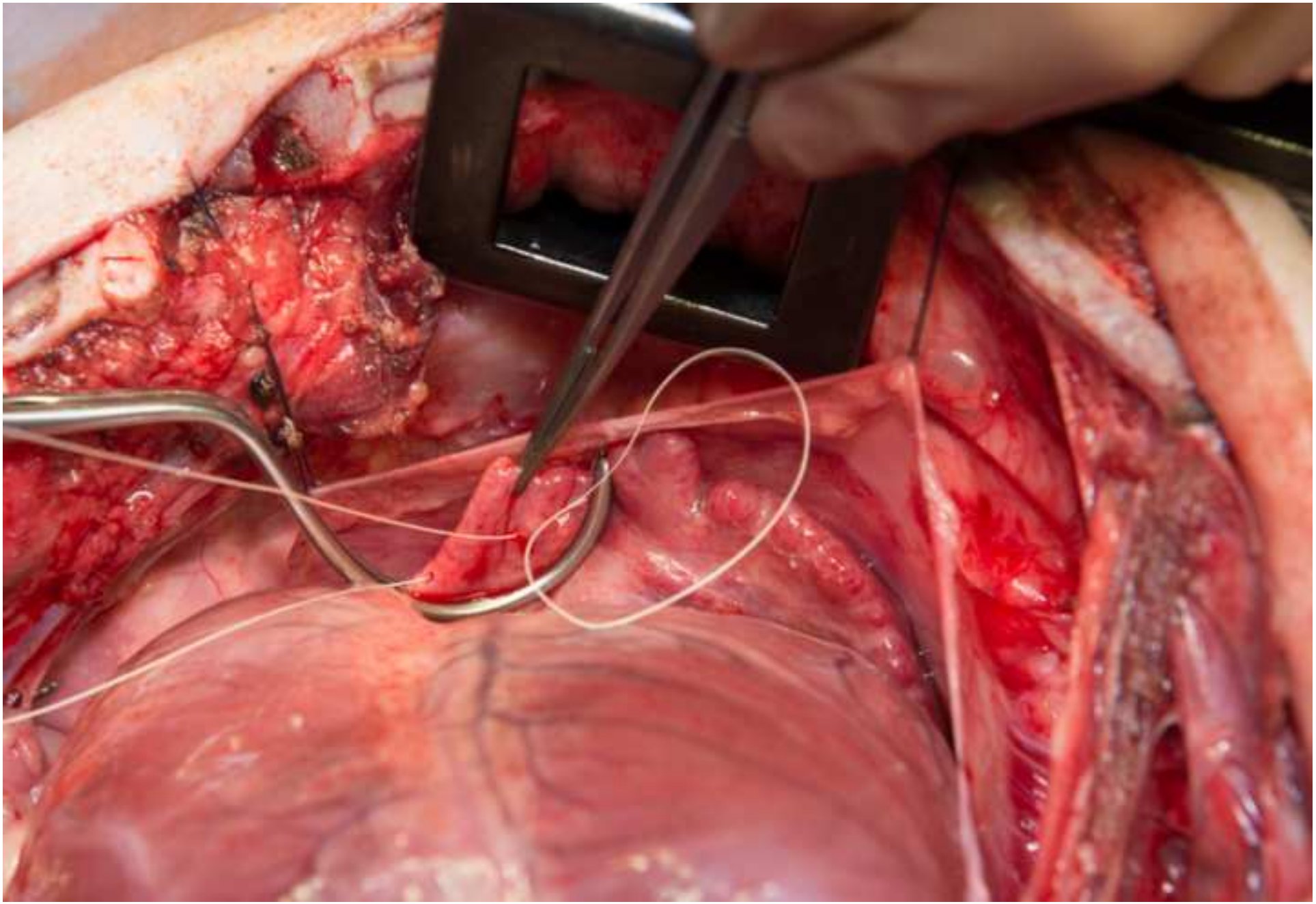

Figure 4

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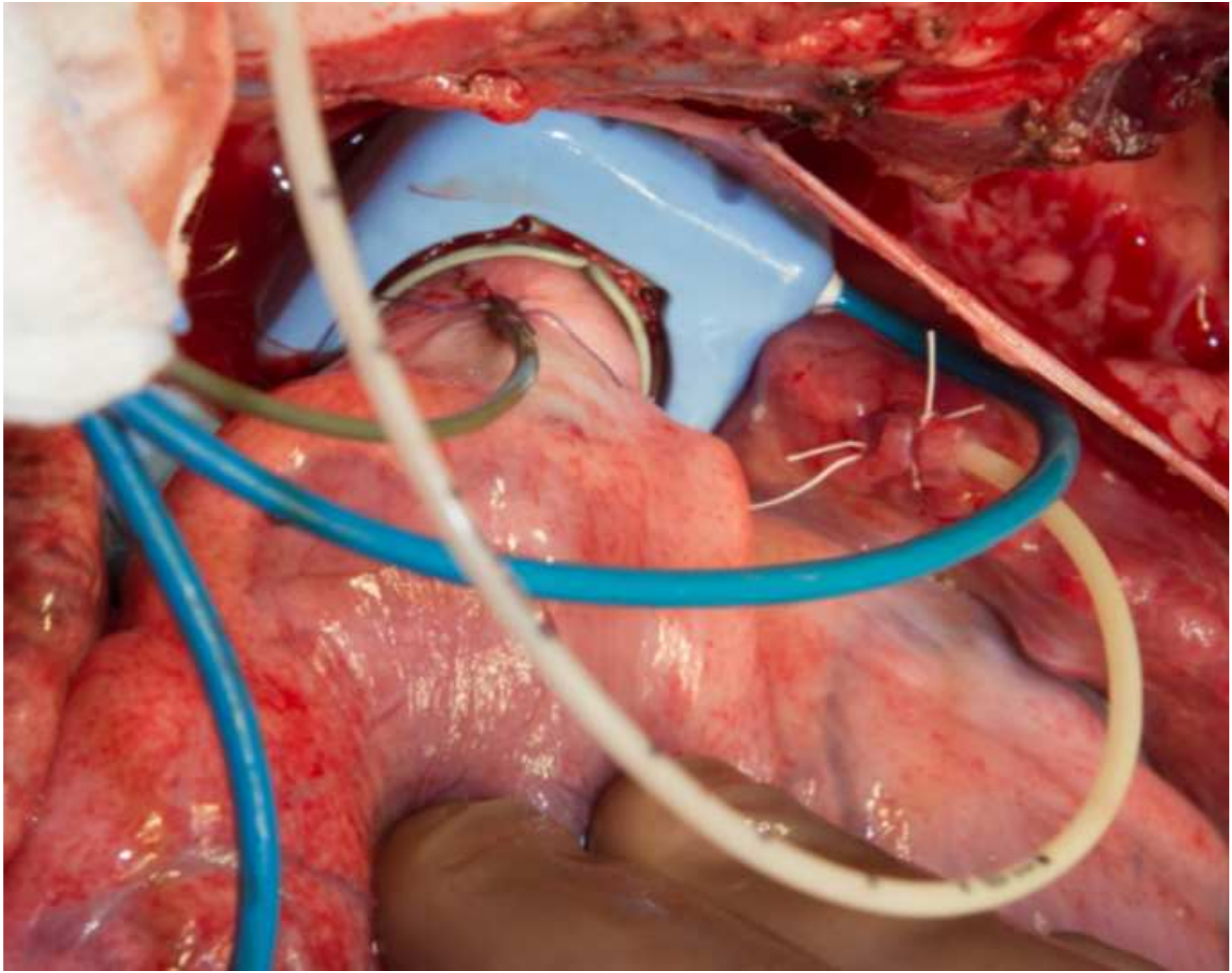

Figure 5

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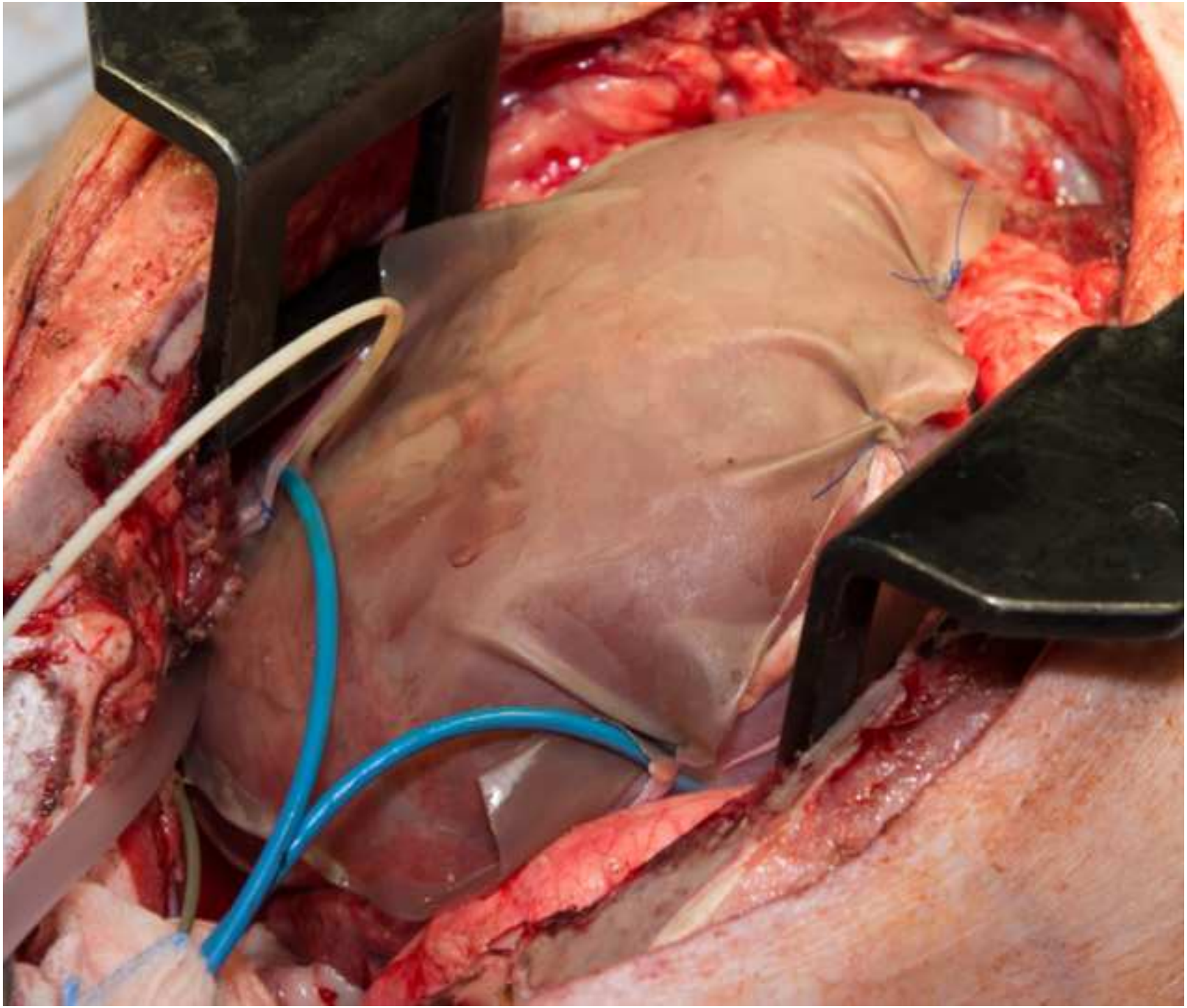

Figure

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Figure 6 arterial pO_2 1 baseline measurement, 2-4 volume loading steps, 5 ARDS

Figure

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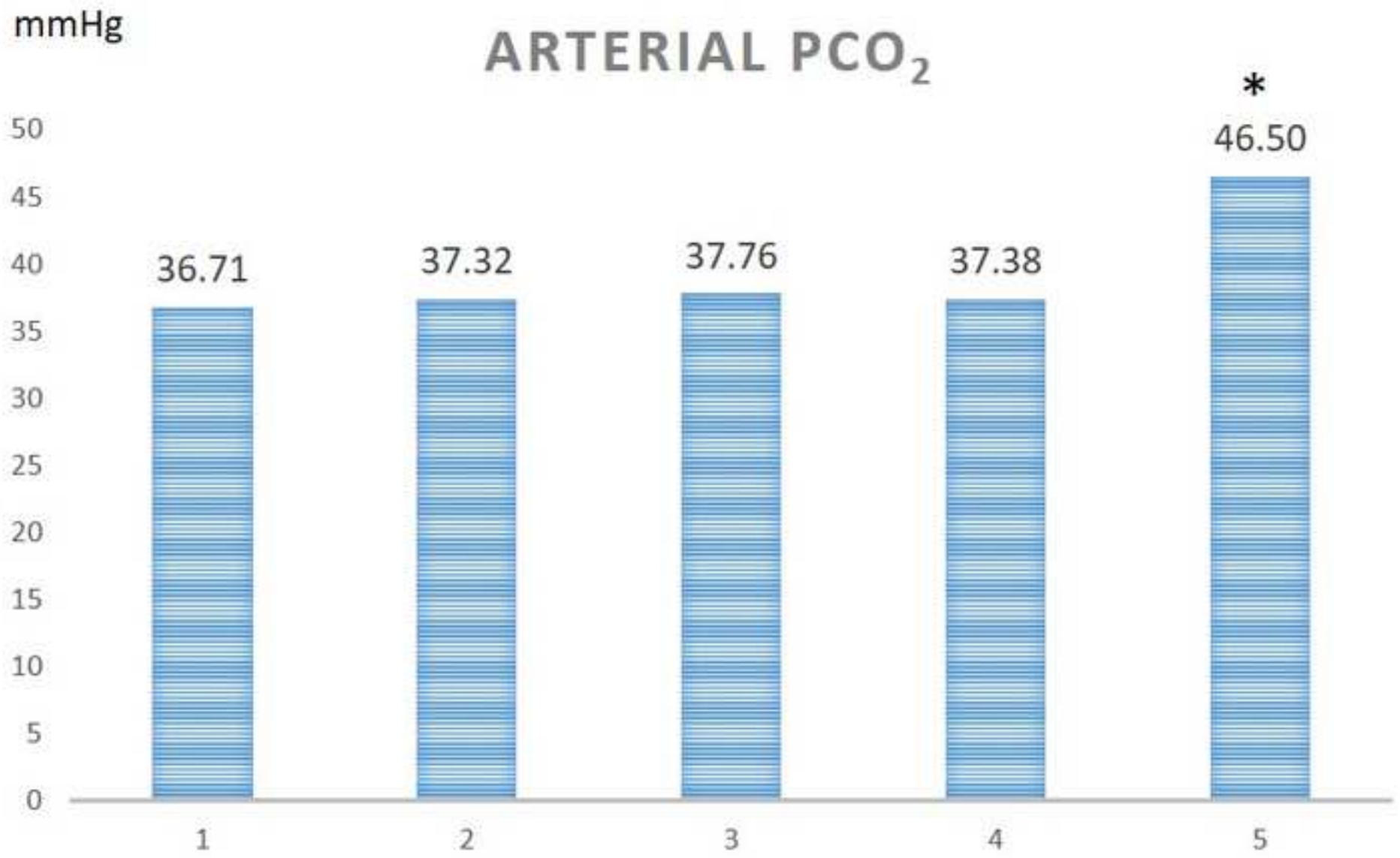

Materials Table

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Name of Reagent/ Equipment	Company	Catalog Number	Comments/Description
Animal Bio Amp	ADInstruments	FE136	
Quad BridgeAmp	ADInstruments	FE224	
Power Lab 16/35	ADInstruments	5761-E	
LabChart 8.1.8 Windows	ADInstruments		
Pulmonary artery catheter 7 F	Edwards Lifesciences Corporation	131F7	
Prelude Sheath Introducer 8 F	Merit Medical Systems, Inc.	SI-8F-11-035	
COnfidence Cardiac Output Flowprobe	Transonic	AU-IFU-PAUProbes-EN Rev. A 4/13	
Adrenalin	Sanofi	6053210	
Oleic acid	Sigma Aldrich	112-80-1	
Magnesium Verla	Verla	7244946	
Ketamin	Richter Pharma AG	BE-V433246	
Azaperon	Sanochemia Pharmazeutika AG	QN05AD90	
Midazolam	Roche Pharma AG	3085793	

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Department: Department of anesthesiology and intensive care Medicine.

Institution: University hospital Hamburg Eppendorf.

Article Title: Invasive hemodynamic monitoring of left and right ventricular Parameters in a pig model of acute right ventricular dysfunction due to ARDS

Signature: [signature] Date: 30.9.17

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Editorial Comments

1. The paper has been revised by a nativ speaker and we hope it is more understandable now,
2. The Figures 6/7 have been changed. We reduced the decimals to two and changed the comma into a period
3. The title, abstract, and introduction are rewritten to be more correct and describe more fully the protocol.
4. At this moment, the blood gas samples as a proof for the induction of ARDS are the only results we can present. The hemodynamic measurement results will be published elsewehere.
5. The additorial comments in the manuscript have been addressed.
6. I will try to upload the figures 6 and 7 as figures.
7. The references have been revised.

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.
done
2. Please provide an email address for each author on the first page.
done
3. Please provide at least 6 keywords or phrases.
done
4. Unfortunately, there are a few sections of the manuscript that show overlap with previously published work. Though there may be a limited number of ways to describe a technique, please use original language throughout the manuscript. Enclosed please find the iThenticate report.
We changed all parts we could.
5. Please add the Short Abstract to clearly describe the protocol and its applications in complete sentences between 10-50 words: "Here, we present a protocol to …". Please attention that in the final version, the Long Abstract will be used as the Abstract. Short Abstract will be used as paper Highlights for the databases.
done
6. Please revise the Introduction to include all of the following:
a) A clear statement of the overall goal of this method
b) The rationale behind the development and/or use of this technique
c) The advantages over alternative techniques with applicable references to previous studies
d) A description of the context of the technique in the wider body of literature
e) Information to help readers to determine whether the method is appropriate for their application
done
7. Please define all abbreviations before use.
done
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done
9. 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. 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.

10. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. The Protocol steps should contain only 2-3 actions per step and a maximum of 4 sentences per step.

11. 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.
done
12. Please leave a blank line between all protocol steps as well as Notes.

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

14. Protocol: What is the age and gender of the animal?
Both gernders, 3-6 month
15. Protocol: 3.1: Please use the imperative tense. Please also add an appropriate reference for proper injection.
done
16. Protocol: 3.2: Please use the imperative tense. How is the step exactly done? Please Clearly describe or refer to appropriate references.
done
17. Protocol: 3.3: How much time is needed for complete anesthesia? How is it checked that the animal is anesthetized?
After sufficient premedication and induction of anesthesia it takes about 10 minutes.
18. Protocol: 3.5: How is the disinfection done? Please clearly describe or refer to an appropriate reference or protocol.
Since we are describing a finalization protocol where the animal is euthanized after the experiment, just cleaning oft he skin is needed. We didn't add any specialized disinfection protocol.
19. Protocol: 4.2: Please use the imperative tense. How is catheter inserted using ultrasound? Please add an appropriate reference or protocol.
we added a few more details
20. Protocol: 4.3, 4.4, 4.5, 5, 6, and etc.: Please use the imperative tense.
done
21. Protocol: 5.2: How exactly is that done? Please provide appropriate references.
done
22. Protocol: 6.1: How are the analyzing steps done? For steps that involve software, please make sure to provide all the details such as "click this", "select that", "observe this", etc. Please mention all the steps that are necessary to execute the action item.
done
23. Protocol: 7.1: How is that done?
We described it in more details
24. Protocol: 7.3: Increase of which CO? Please clearly describe the steps.
We described it in more details
25. Protocol: 8.2: How is that done? Please describe or refer to an appropriate protocol.
We described it in more details
26. Protocol: 8.3: How much adrenalin? How is that administrated?
We described it in more details
27. Protocol: 8.4: Add to what? How?
We described it in more details
28. Protocol: 9.1: Which parameters? How? If referring to other steps, please mention. Please clearly describe the steps or refer to appropriate references.
We described it in more details
29. After revising the protocol, please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.
done
30. Please discuss all figures in the Representative Results. However, for figures showing the experimental set-up, please reference them in the Protocol.

done

31. Please include a title and a description of each figure and/or table. All figures and/or tables showing data must include measurement definitions, scale bars, and error bars (if applicable). Please include all the Figure Legends together at the end of the Representative Results in the manuscript text.
done
32. Each Figure and Table Legend should include a title and a short description of the data presented in the Figure and relevant symbols. The Discussion of the Figures should be placed in the Representative Results.
done
33. Please upload each Figure individually to your Editorial Manager account as a .png, .pdf, or a .tiff file. Please combine all panels of one figure into a single image file.
done
34. Table 1 and Table 2: If they are tables, please upload them individually in the form of an .xls or .xlsx files. If they are plots, please upload them individually as a .png, .pdf, or a .tiff file. Please include the labels with unites for both axes.
We changed it into figures.
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No figures from previous publications were used.
36. Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials in separate columns in an xls/xlsx file.

Reviewers' comments:

Reviewer #1:
Major Concerns:
None

Minor Concerns:
None

Reviewer #2:
We have read with interest the manuscript entitled "Invasive hemodynamic monitoring of left and right ventricular parameters in a pig model of acute right ventricular dysfunction due to ARDS".
The authors set up an animal model aiming to comprehensively monitor the development of right ventricular failure (RVF) after induction of ARDS with infusion of oleic acid.
The purpose of their experiment is clearly stated. Indeed, the best hemodynamic framework to assess right ventricular function within this context is yet to be established. Clinical benefit was clearly delineated as well.
Experiment protocol is detailed and reproducible. A very inclusive data gathering system is to be expected; we envisage that simultaneous RV and PA pressure recordings may serve to

infer load-adaptability and RV diastolic pressure metrics.

Minor concerns:
We fully agree with the authors' comment on CVP as a marker of volume state and fluid responsiveness. Assessment of preload recruitability of stroke work (PRSW) is essential within any circulatory shock. This is even more crucial when facing RVF as unwarranted fluid could lead to parallel (RV-LV) uncoupling. We note that volume optimization of animals is performed until PRSW is worn out ("continue until there is no more increase in CO of more than 10 %"). It is therefore implicit that within an increased right ventricular afterload, fluid unresponsiveness is a self-fulfilling prophecy. This detail does not negate the value of the model but we think it should be underlined. After all, this will be a model of RVF which starts from a point where further fluid would already be unjustified and potentially deleterious. We may understand that this tactic is the authors' way to set up a common denominator/baseline amongst studied animals. Whatever the reason, we feel that this is worth being explored/explained.
We commend the authors for a valuable animal model which will yield a comprehensive hemodynamic overlook at RVF. It is an essential component of an integrated approach.

The reviewer is right. We used this setup to have a baseline animal model for measuring hemodynamics in ARDS. After baseline measurements after inducing ARDS in stable hemodynamics, we further investigated hemodynamics in this model after reducing and replacing volume load in animals. These results will be published in a paper for hemodynamic monitoring, since we just described our methodology here. We added a note in step 9.2 that one can use this model for further investigating volume load like we did.

Reviewer #3:
Manuscript Summary:
Authors described a set up for comprehensive hemodynamic monitoring in a model of right ventricle dysfunction secondary to ARDS caused by oleic acid. I think that this set up could be interesting for this purposes, however I have the following concerns about the protocol and description made by the authors.

Major Concerns:
- Actually, after reading the introduction I cannot really understand what the major focus of the authors is. They start talking about right ventricle and ARDS but at the end they talk about fluid responsiveness. Both topics are really important and maybe related but authors should better describe what they exactly want to describe with the set up, protocol and study.
- Flow probe calibration should be better explained. Maybe a picture of the calibration screen would work.
We don’t have any animal studies planned until December 27th so it is not possible for us to add a picture until our deadline. Our next pig model using a Flow probe starts January 10th. If a picture is required we could late file it.
What do the authors mean with "mark an area with 10l/min"? Is this a real flow measurement or a preset value for calibration that the software provides? I am very familiar with labchart and blood flow measurement with transonic probes and do not understand what authors mean.
This is used as a preset value.
- About the pressure calibration, the authors did a calibration between 0-100mmhg but systemic arterial pressure can be above 100mmhg. Did the sensor maintain linearity above 100mmhg making the calibration adequate? Please explain.

We added an explanation in 2.5

- According to the order that the authors followed in the description there is not any monitoring or venous access during the tracheotomy. Is this safe enough for the animal?

The ECG stickers and the oxygen probe on the tail were put on right after the premedication was done, before starting the tracheotomy. We put it in the right order.

- What was used the PAC for? The authors mentioned that the PAC can introduce problem the flow measurement; however they described that they did inserted a PAC and also inserted a 8f sheath for the PAC (4.2 and 4.3). In case they remove the PAC, How did they measure mixed venous blood gases?

As described in our setting, we put the PAC into the right ventricle (RV) and not the pulmonary artery (PA) beacuse this could interfere with the flow measurement oft he flowprobe around the PA. Mixed venous blood gases were taken by inserting the PAC a little further into the PA and pull it back into the RV after measurement.

- Authors described that before opening the chest 8 mg pancuronium iv was given, but they did not mentioned how they assured that anesthesia and analgesia is adequate or if ti was modified for the sternotomy. Actually neuromuscular blockers make exploration of pain so hard in animals.
- There is no description about how they choose the adequate size of the flow probes.
- There is no description about how the CVP was acquired or measured.
- I cannot understand the volume loading protocol: 7.2 "If the cardiac output does not increase at least 10% start another volume loading step" and 7.3 "Continue until there is no more increase in CO of more than 10%." ???

We described it in more detail

- 8.1 "Increasing the FIO2 to at least 0.5 to 0.8 as required." As required according to what?

We added a spO_2 of 90% as required oxygen saturation.

- 8.3 "Using continuous administration of adrenalin to keep hemodynamics stable". What criteria did the authors follow to define hemodynamic stability?

We did that.

- 8.4 "Adding Calcium, Magnesium and antiarrhythmics (Lidocain 1%) as required during the infusion of OA" What criteria did the authors follow to use this?

Since hemodynamic monitoring in this setting is dependant on a stable sinus rhythm, we added calcium and magnesium whenever the level were lower than normal values and lidocain was added if there was still no stable sinus rhythm.

- Title number 9 is "Volume optimizing" and in 9.1 authors say "After induction of mild to moderate ARDS, another measurement of all parameters is done (M 2)." Does this mean that authors infused volume again according to their criteria and then performed another measurement? If so, please mention it in the text.

We did

- Line 185 "Results of previous OA induced acute lung injury (ALI) models were inconsistent" What do authors mean with inconsistent? Inconsistent about what? The rest of the paragraph did not describe any inconsistency.

We added the information about this.

- No description about the evolution of the measurement that the authors obtained with the whole set up (pulmonary artery pressure and flow, aortic pressure and flow, picco, etc.) is given. This is really disappointed as the authors are proposing their set up as an ideal one to have all these measurements simultaneously.

As discussed with our supervisor Nandita Singh before submitting the article, we made sure that our hemodynamic results are not subject of this paper. This is just about the methodology. The results of our measurements and our findings will be bublished seperately.

Also the authors mentioned in the abstract that CVP did not change and made an statement about volume status. Actually CVP can increase after ARDS as a result of the right ventricle dysfunction.
In our setting of acute right ventricular dysfunction with a duration of about 3-5 hours, we found no increase in CVP. Compared to chronic right ventricular dysfunction which goes along with an increase of CVP:

Reviewer #4:
Manuscript Summary:
The manuscript describes how to monitor the pulmonary artery pressure and the left atrial pressure with high fidelity catheters inserted directly in these structures through a sternotomy; how to monitor the arterial flow in the ascending aorta and in the pulmonary artery trunk with flow probes disposed around these vessels; how to measure hemodynamics with a PICCO and a Swan-Ganz catheter and blood gas variations in pigs during induction of an acute respiratory distress syndrome (ARDS) with intravenous oleic acid injection.

Major Concerns:
There are many major concerns.
- First, the title of the article does not fit with the content: the title announces left and right ventricle parameter evaluation in a large animal model of ARDS, whereas there is no evaluation of left and right ventricular parameters.
The article mainly describes how to monitor global hemodynamics (cardiac output, systemic pressure, right atrial pressure) and right ventricular afterload (pulmonary artery pressure and left atrial pressure). Evaluation of left and right ventricular function would at least require echocardiography, pressure volume-loop analysis, biomarkers of ventricular distension or lesions.
We did echocardiography and took blood samples in out study the results and hemodynamic finding will be presented seperately. We decided to spare out the echocardiography in this paper, because it would be a whole new paper. To take blood samples as required for further experiments should be easily done with all the venous and arterial lines in place.
This paper is a methodoly paper where we are trying to describe the method to gain all hemodynamic parameters. The script and video should help others to reproduce the experiments.

- Second, the objective of the article is unclear, is it to establish a model of acute respiratory distress syndrome, or to describe hemodynamic monitoring, or both? The model of ARDS with oleic acid have been largely described in the literature, as well as hemodynamic monitoring using flow probes around vessels and high fidelity catheter. However, hemodynamic evaluation of the model is interesting, and the description of the feasibility of invasive hemodynamic measurement is important to the field. In this objective, authors should report hemodynamic measurements which were not reported in the manuscript. The authors should provide evidence of the efficiency of the method they are describing (i.e. pulmonary and systemic pressures, atrial pressures, cardia output with both PICCO and Swan-Ganz methods; some right ventricular parameters derived from right ventricular pressure signals such as dP/dtmax, dP/dt min and TAU).
As said before we are just describing the methodology here. Our findings and hemodynamic parameters will be presented elswhere.
- Third, wrong affirmations and not inaccurracies:
In the abstract line 15-18: cardiac hemodynamics is not the same than aortic and pulmonary hemodynamic.

line 21-22: in the listing of systemic hemodynamic parameters, RVP (right ventricular pressure) is not a systemic parameter, it is a right ventricular parameter, furthermore it should be precised which RV pressures (pic systolic, end systolic, end diastolic) .
With this methodology it is possible to detect peak systolic, end systolic and end diastolic right ventricular pressure using the Labchart 8. However, as said before the hemodynamic findings were not part of this paper.
line 31: "we were able to confess" is inadequate to express a conclusion; the conclusion "the CVP might not be the right tool to monitor volume status..." is not supported by the data and the study was not designed to answer this question.
Thank you. We changed that.

INTRODUCTION
-line 39: I agree with the lack of isovolumic relaxation phase in the right ventricle under physiological conditions due to the decrease in right ventricular pressure during ejection. However, the statement "the absence of isovolumic phase of contraction" is wrong, there is a phase of isovolumic contraction in the RV.
This line has been changed.
line 45: "right ventricular pressure parameter like central venous pressure" is wrong, here there is a confusion between right ventricular parameters and central venous pressure (CVP), CVP is not a RV parameter. There is a global consensus to say that right atrial pressure elevation is a part of the acute right heart failure syndrome with the decrease in cardiac index. Here, there is a confusion between the acute right heart failure syndrome and right ventricular parameters.
-lines 51-53: the article does not answer the main objective ==> The article describes invasive hemodynamic monitoring of aortic and pulmonary artery hemodynamics in a large animal model of ARDS. The statement "in right heart failure" suggests that the authors should define what is right heart failure in their study. There is not consensus about a quantitative definition of right heart failure; most authors consider that a right atrial pressure above 8 mmHg and a cardiac index < 2.2 L/min/m2 defines right heart failure; for qualitative definition of acute right heart failure, see consensus statement by Harjola et al. 2016.

PROTOCOL
-line 69: usually Millar recommend to warm up the catheter to the animal temperature to avoid shift in pressure measurement.
Thank you. We added the term „warm“ water to our protocol.
-line 16o: this is ARDS induction and not right heart failure induction.
We changed this.
-Line 194: Table 1 is not a table, this is a Figure.
-Line 196: Table 2 is not a table, this is a figure.
This is right. As from my correspondance with Nandita Singh from October 1st I told her that I was not able to upload this table as a figure. She answered that she would change it and put it in the right order once I sent it to her.
-line 206: the jugement millar offers consistent measurements, this is OK, but fluid filled catheters also (most used method in clinical practice)... Millar offers high fidelity measurements of pressure and is the gold standard, but fluid filled catheter are "consistent", there is of cours limitations (damping, zero placement, removing air from the lines...).
This is why we used both methodes, Millar catheters and for example PICCO and PAC.

Minor Concerns:
-line 185: "results of previous OA induced ALI models were inconsistent" should be avoided;

it is unlogical with the next sentence "...infusion of OA is an easy and good model to induce ARDS..."; Please site references.
We added a few more references
-line 196: please define the Horrowitz index.
We changed it to oxygenation index (Horrowitz Index) because this is more commonly known in the english language.
-line 222: dissected may be replaced by divided.
As a cardiac surgery resident I have to say that one has to dissect the PA from the Aorta using scissors because there is a lot of connective tissue that combines both. It is not just easily divided.

In conclusion, the topic is interesting since hemodynamic evaluation of ARDS is central in clinical management of patients with ARDS. However the focus of the study is unclear as there is a lot of confusion between right ventricular parameters, hemodynamic parameters and right heart failure. The authors should precise the main objective of their work and provide methods and results that fit better to the question and the data.

Reviewer #5:
The authors provide a description of a pig model to assess hemodynamic changes, especially with regards to pulmonary artery pressure and flow and right heart funcion, during induction of ARDS using oleic acid injection. It seems to be a complete model, but not enough data is given to assess if it is feasible.

Major issues:
Introduction
In naming the measured parameters, please be consistent with regards to naming convention used. For example, in two parameters the term „flow", and in another „output" is used to describe the same physical property.
Protocol
Please give a table listing all measured parameters and their correct units.
Since all the equipment is in place, one might consider to measure pulmonary artery PPV.
With our equipement beeing in pkace the PA PPV is one oft he main parameters we were investigating. Our results will be published soon. It is easy to calculate when all catheters are in place.
Outcome
On what grounds is the conclusion based that the model is „feasible to show a broad variety of hemodynamic parameters"?
You demonstrate that arterial oxygenation decreases and carbon monoxide increases. Please provide data about the hemodynamic changes to show if the pulmonary changes induced are reflected in the hemodynamic measurements.
Since this paper is about the methodology, our data about hemodynamic changes in ARDS will be published soon.

Minor issues:
Abstract
line 28: I suggest „induce pulmonary hypertension" instead of „create a pulmonary hypertension"
The term „to induce acute respiratory distress syndrom“ is used many times in literature. It is also the title of papers we are are citing.

1. Akella A, Sharma P, Pandey R, Deshpande SB., Characterization of oleic acid-

induced acute respiratory distress syndrome model in rat. Indian J Exp Biol. 2014 Jul; 52(7):712-9.

2. Zhu YB, Zhang YB, Liu DH, Li XF, Liu AJ, Fan XM, Qiao CH, Ling F, Liu YL., Atrial natriuretic peptide attenuates inflammatory responses on oleic acid-induced acute lung injury model in rats, Chin Med J (Engl). 2013 Feb; 126(4):747-50.

Protocol

There are many kinds of Millar catheters. Please give more specific information about the system used.

We used the Millar SPR-350S and added this information in the manuscript.

Table 1

Use a meaningful number of significant figures! A graph like this should be used to give some information about the data than the mean. Is the data normally distributed? If so, please give error bars (+- values are given in the text, do they describe standard deviation?). Or, consider using boxplots. I salso uggest to use the term „oxygenation index".

We changed it to „oxygenation index“ and put marks on the graph.

Table 2

See comments to Table 1. Also, Table 2 should be combined with Table 1 since they describe two related aspects of the same process. The information