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

8. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.

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.

35. If you are reusing figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [AUTHOR] et al.[REFERENCE]”.

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 spO2 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