Peer Review File
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Reviewer A

Comment 1: How do you propose assessing lungs in DBD donors without lung recruitment prior to aortic cross clamping? The basis for retrieval in DBD donors rests on adequate recruitment and suitable ABG's which obviously cannot be performed if your technique is adopted.
Reply 1: In general, the usability of lungs is adequately verified using multiple-donor clinical parameters such as donor's background information, laboratory assessments, bronchoscopic and radiological findings, gross inspection, and those transitional changes in the process. In addition, EVLP could be also an alternative powerful tool. Although we believe that the decision can be safely made by an experienced surgeon even if a gas analysis under lung recruitment is unavailable, the technique we explored in this study is not accordance with the recommendation of the ISHLT consensus document as the reviewer pointed out. Therefore, in Discussion, we cautiously mentioned the potential risk for the assessment in this technique and proposed necessity of EVLP as needed.
Change in the text: We described about the issue in Page 14, Line 243-249 with some additional text.

Comment 2: You also mentioned storing the excised heart lung block at 4 degrees Celsius- how was that achieved? Was the temperature set at 4 degrees or the perfusate temperature/organ temperature measured?
Reply 2: The excised heart lung block was stored in the refrigerator set at 4 degrees Celsius until implantation. The perfusate temperature and organ temperature were not measured.
Change in the text: We have modified our text as advised. (See Page 7, Line 116-118)

Reviewer B

Comment 1: They should justify why they have used 3 animals in the control group instead of 5.
Reply 1: The key part of this study is to compare the AT-ACR to AT-CR groups. The control group was served to verify that there was no procedural problem. Since it was expected that the control group would perform better, the sample size of the control group was reduced to minimize

the number of involved animals from an ethical perspective.
Change in the text: We added a text as advised. (See Page 5, Line 85-86)

Comment 2: A complete atelectatic lung is usually not the case during a procurement. They should discuss the clinical relevance of this inflammatory reperfusion response in a case of a sublobar atelectatic lung.
Reply 2: We applied a completely atelectatic donor model to avoid letting an inflated part of lung affect the experimental results and look at the sheer effect of ACR on the atelectatic donor lungs. Basically, we consider that sublobar and total lung atelectasis share the similar detrimental pathology in reperfusion injury at the affected regions. In addition, we rather believe that the ability to set up such extreme conditions is one of the advantages of animal experimental study which can experimentally focus on the objective factor. As the reviewer claims, however, there could be a potential pathophysiological difference in the experimental intervention between total and partial atelectasis. We provided a caution for this issue as a study limitation.
Change in the text: None. We have described about this problem in Page 14, Line 253-256.

Reviewer C

Comment 1: The histological findings and the lung injury scores of the graft tissue at the end of the evaluation need to be presented in tables or graphs with the full specific data. It was described that less intra-alveolar edema, neutrophilic infiltration and hyaline membrane formation was observed in the AT-ACR group than in the AT-CR group, but this data was not showed.
Reply 1: I see your point.
Change in the text: As the reviewer advised, Figure 4 was modified and newly designated as Figure 6.

Reviewer D

Comment 1: Aortic clamping with or without cardioplegia / topical cooling of the heart? When did the investigators start the alveolar recruitment? This should be better explained in the methods of the paper and illustrated in Figure 1.
Reply 1: Topical cooling of heart and lung with crashed ice and cutoff of superior and inferior vena cava were performed at the same timing as aortic clamping. These procedures resulted in prompt cardiac arrest and removal of blood from right heart to prevent from perfusing pulmonary artery and lung. In AT-ACR group, alveolar recruitment was performed following aortic clamping

and prior to start cold flushing, in acirculatory and normothermic states as described in Page 7, Line 109-111 and Figure 1.
Change in the text: We added text as advised (See Page 7, Line 113-116) and modified Figure 1.
Comment 2: Pulmoplegia also creates a circulation through the lungs:
Lungs were cold-perfused with LPDG solution. Pulmoplegia, however, also creates a circulation through the lungs. So in fact, the terminology currently used for the study group "AT-ACR" is not entirely correct as these atelectatic lungs were also "recruited" prior to re-circulation (not with blood but with cold crystalloid preservation solution)! Please consider to use another (different from CR versus ACR) abbreviation for both study groups.
Reply 2: Cold flushing (pulmoplegia) was conducted equally in the CR and ACR group as a common preservation technique. This process was performed not during but after lung recruitment for both the group (Please see Figure 1). Therefore, preservation solution was not perfused during the recruitment procedure in both the groups. But it is certain that the terminology for the study group could be revised to represent the study condition more precisely as reviewer suggested. We would like to modify the group name from CR/ACR group to "blood-circulated recruitment (BCR) /no-BCR group".
Change in the text: We changed abbreviation for study group in modified the Figures.

Comment 3: Please elaborate in the discussion on the possible mechanism for the observed outcome differences between both "(re)circulation" groups (warm blood perfusion group versus cold crystalloid perfusion group).
Reply 3: Our hypothesis for the mechanism and pathology was described in Page 12, Line214-227. We consider that the lung injuries provoked after blood-circulated recruitment procedure examined in this study mimic the phenomenon found in re-expansion pulmonary edema (RPE). Several studies have reported that inflammatory and pro-inflammatory cytokines play important roles in the development of RPE. We described about this in the discussion.
Change in the text: We have discussed the potential mechanisms for the study results in Page 12, Line 214-227 with some additional text.

Comment 4: What about hemodynamics in the transplant recipient. Was there any difference in pulmonary flow through the transplanted left lung (before and after clamping of the right pulmonary artery) between both study groups in terms of pulmonary vascular resistance, pulmonary artery pressure, pulmonary flow, and cardiac output? Please provide additional data for post-transplant hemodynamics in all groups.
Reply 4: Certainly, hemodynamics is important, especially in transplanted left lung measuring with clamping of right pulmonary artery. But post-transplant cardiac output was lower than

baseline. Especially after clamping of right pulmonary artery, valid values were not available under stable conditions in some cases. Therefore, we cannot provide sufficient data about cardiac output, pulmonary flow and pulmonary vascular resistance. We described additional data for post-transplant mean left pulmonary artery pressure under the condition of clamping of right pulmonary artery.

Change in the text: As the reviewer advised, we added additional data (See Page 10, Line 178-179) and Figure 2 was modified and newly designated as Figure 3.

Comment 5: The reviewer would suggest to modify the title as follows: "Alveolar recruitment after cardiac arrest during procurement of atelectatic donor lungs is a protective measure in pulmonary transplantation"

Reply 5: Drawing on the reviewer's suggestion, we modified the title as follows: Lung recruitment after cardiac arrest during procurement of atelectatic donor lungs is a protective measure in lung transplantation

Change in the text: We changed the title as above. (See Page 1, Line 2-3)

Comment 6: Please include some macroscopic images of both study groups.

Reply 6: I see your point.

Change in the text: We added a figure of the gross findings as the reviewer recommends. (See Page 6, Line 105, Page 10, Line 172-174 and Figure 2)

Minor Comments: Grammar/typo's - please correct:

- line 110: "low-potassium-dextrin-glucose solution"; should be "dextran"
- line 181: "… different from the levels in the AT-CR group"; should this not be the "AT-ACR" group??
- line 209: "…. the development of ischemic reperfusion injury"; better "ischemia-reperfusion injury"
- line 249: "…potentiality …"; this should be "potential"

Reply: thank you for pointing our error.

Change in the text: We corrected these errors. Additionally, some values were not aligned with the number of significant digits, so they were unified.