**Author’s response to reviews**

**Title:** EFFECT OF PEEP AND I:E RATIO ON CEREBRAL OXYGENATION IN ARDS: AN EXPERIMENTAL STUDY IN ANESTHETIZED RABBIT

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EFFECT OF PEEP AND I:E RATIO ON CEREBRAL OXYGENATION IN ARDS: AN EXPERIMENTAL STUDY IN ANESTHETIZED RABBIT
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BMC Anesthesiology

Dear Dr Tu,

Thank you for giving us the opportunity to submit a revised version of our manuscript entitled “Effect of PEEP and I:E ratio on cerebral oxygenation in ARDS: an experimental study in anesthetized rabbit”.

We are thankful to the reviewers for their careful assessment of our data. We have done our best to respond appropriately to all of their issues and concerns. I am uploading a point-to-point response to their comments.

We hope you find our manuscript suitable for publication look forward to hearing from you.

Sincerely,

Prof. Sam Bayat MD PhD
The research abstract is very well writing and refers to a very interesting topic on the field of mechanical ventilation and ARDS. The results are very interesting and well described. However I have few comments, please find it below:

# 1:
Line 38 of Materials and methods:
Please rephrase the following sentence: "… resulting in a tidal volume (VT) of approximately 6 ml/kg." or replace it on line 35, the way it is sounds as if the adjustment of the RR has an influence on the amount of the VT generated.

R1. The sentence was corrected. Thank you.

# 2:
There are different approaches to adjust the PEEP level very well described in the literature e.g. according with the compliance. Please justify why the PEEP levels of 6 and 9 cmH2O was used in the current work and justify why the authors decided not to use one of the approaches already described in the literature.

R2. We agree with the reviewer that a number of approaches have been described to set optimal PEEP based on respiratory mechanics. The goal here, was to assess the effect of both PEEP and I:E ratio, on brain and tissue oxygenation. Setting different PEEP levels in each animal would have considerably complicated the interpretation of the findings, and particularly the effect on hemodynamics and gas exchange. We there chose to compare a low and a high PEEP level.

# 3: I am particularly curious why the authors used forced oscillation technique to measure respiratory mechanics. Considering that the animals were ventilated using control mode, it would be easier to apply the equation of motion and make the estimation of respiratory mechanics variables.

R3. The reviewer is correct that flow and pressure signals can be used to compute the overall respiratory system mechanical properties. The rationale behind using oscillometry was a better partition of airway vs. lung tissue mechanics.

Pablo Cruces (Reviewer 2): I read with interest the manuscript entitled "EFFECT OF PEEP AND I: E RATIO ON CEREBRAL OXYGENATION IN ARDS: AN EXPERIMENTAL STUDY IN ANESTHETIZED RABBIT"
ANESTHETIZED RABBIT. Although the study has some potential relevance, I have major concerns -see the enclosed comments. Serious confounding methodological issues must be analyzed. Note that substantial revision will be necessary before publication can be reconsidered. I have several specific comments, which are listed below and you may consider for improving your manuscript.

INTRODUCTION
1. Paragraph 1: It is necessary that the authors base more deeply the need to use (and study) reverse radio ventilation. References mentioned are more than 20 years old. Hemodynamic risk of auto PEEP is not mentioned, which is very relevant in the context of evaluation of oxygenation and cerebral perfusion.

R1. We are now citing 2 recent references, including one of IRV in general anesthesia of abdominal surgery patients, which underscores the relevance of our data. We agree with the reviewer regarding the hemodynamic risk associated with IRV. This is mentioned in the first sentence of the second paragraph.

2. Paragraph 2: The prevalence of cognitive impairment in ARDS has not been studied in patients ventilated with IRV.

R2. We agree with the reviewer that data on cognitive outcome specifically in ARDS patients ventilated with IRV is lacking. However, our point here is that since there is both evidence of cerebral autoregulation impairment, and that of cognitive impairment in patients with ARDS, whatever the ventilation mode, understanding the impact of PEEP and ventilation modes such as IRV is an important question. Also, we argue in the discussion that other ventilation modes such as APRV, which is an inverse-ratio ventilation mode, is intensely used, including as the primary mode in ARDS in some centers. This mode bears similarities with IRV in that mean airway pressure is increased through a prolonged inspiratory time, without increasing peak airway pressure.

MATERIALS AND METHODS
3. I'm not clear if the subjects were euvolemic. Was the preload evaluated? The fasting time prior to the experiments is not mentioned.

R3. The reviewer raises an important point. We measured CVP which was above 8 mmHg at baseline. The MAP was normal and did no oscillate over the respiratory cycle. Also, the animals were not fasted and they received an infusion of lactated Ringer’s solution (4 ml/kg/h) after an initial bolus of 0.5 to 1.0 ml per kg of hydroxyethyl starch at the time of anesthesia induction. Therefore, according to our data, the animals were euvolemic.

4. It would have been relevant to measure cardiac output, to determine if changes in perfusion and cerebral oxygenation are secondary to changes in CO.

R4. We fully agree with the reviewer. Unfortunately, this was not technically possible. However, we believe that the drop in MAP and CVP at baseline both suggest a drop in cardiac output. We have added this point in the study limitations section of the discussion.

5. There is a lack of information on the manufacture of drugs used.
R5. Information on the manufacturer was added for all drugs in the Methods section.

5. I do not understand why not use volume control mode, which would have simplified lung mechanics measurements. I'm not sure how they measured intrinsic PEEP without performing an expiratory pause.

R5. Volume control mode was not used, because this ventilator mode is seldom used in patients with ARDS, because of the risk of ventilator-associated lung injury. Intrinsic PEEP is suggested by the slight but significant increase in the minimal airway pressure under IRV, as compared to an I:E of 1:2 (see: Figure 1).

6. The authors used a 3-hit model of ARDS (LPS / surfactant depletion / VILI). Why?

R6. Models of lung injury with whole lung lavage and surfactant depletion only are notoriously recruitable, and are often criticized for not closely resembling the clinical situation where ARDS results very often from multiple insults, including inflammation and exaggerated mechanical stress due to mechanical ventilation. We believe therefore that our ‘multiple-hit’ model is a better representation of clinical ARDS.

7. Authors do not explain what is the relevance of measuring "muscle oxygenation indices".

R7. We concomitantly measured muscle oxygenation to assess whether the observed changes in brain oxygenation result from local or systemic processes. For example, in injured lung on PEEP 6 mmH2O, both brain and muscle oxygenation are reduced, likely as a result of a severe overall hypoxemia. Therefore, here the effect on tissue oxygenation is generalized and not exclusively cerebral. We added a clarification in the methods section.

8. Also you must state whether a sample size calculation was conducted. Despite the reason mentioned by authors, I believe that using information on known changes in tissue perfusion and oxygenation induced by IRV.

R8. We take the reviewer’s point that based on our current data, a sample size can be computed. Actually, all of the statistical tests were performed with adequate power. What we are stating in the Methods section of the manuscript however, is that no formal sample size calculation could be performed ‘a priori’, due to lack of required data. We are not sure whether an ‘a posteriori’ calculation of sample size is useful, since we have computed statistical power for all performed tests.

9. Please add if a randomization method was used.

R9. As stated in the Methods section (page 7), the order of I:E ratios was randomized.


R10. As stated in the Methods section, the animals were placed on a thermostatic heating pad and internal body temperature was maintained at 38–39°C, which is normal for rabbits.

RESULTS

11. The respiratory rate is not mentioned under different conditions. As I understood the methods, the
respiratory rate was adjusted according to ETCO2 target. However, in conclusions it is mentioned that the respiratory rate was constant. Please explain.

R11. We apologize for the lack of clarity. Respiratory rate was adjusted only at baseline to maintain normal exhaled CO2. This is now clarified in the Methods section.

12. Is it possible to affirm, using CVP, MAP and CF, that "increasing the I:E ratio decreased venous return"?

R12. The statement was revised. We take the reviewer’s point that it would have been better to measure cardiac output and agree with the reviewer that CVP is an imperfect indicator of volume status, however, it does seem to vary with hypovolemia and fluid resuscitation in rabbits (see: Chen et al. J Trauma. 2009 Mar;66(3):676-82). We have added a comment on this point in the study limitations section of the Discussion.

13. Figure 2: Imperfect legend.

R13. The legend was corrected. Thank you.

14. FIO2 was 100% in subjects injured. Why PaO2 differs from PaO2/FIO2?

R14. Please note that FIO2 was 100% only during the injurious ventilation phase. Thereafter, the FIO2 was reduced to 0.9. This is now clarified in the Methods section.

Stefan Kreyer (Reviewer 3): Dr. Lovisari and Co-workers present in this work their data about the effects PEEP-Level and Inspiration : Expiration ratio on cerebral oxygenation in healthy lungs and in an experimental ARDS. Their work focuses on different parameters to measure cerebral oxygenation. The authors chose an experimental ARDS in rabbits by intravenous LPS injection and saline lavage. The authors could show that IRV decreased cerebral oxygenation, while in ARDS IRV did not improve cerebral oxygenation, despite improving gas exchange. The increase in PEEP did improve cerebral oxygenation despite lower CF. Data presented here are interesting, although some questions arise.

Major concerns:

1. Animals in PEEP6 are extremely hypoxic. This level of hypoxia may influence cerebral autoregulation and cardiac function, which may create a major bias. How do you exclude this bias.

R1. We agree with the reviewer that severe hypoxic and hypercapnic conditions may affect cerebrovascular autoregulation. Please note that we took care to perform the PEEP 6 measurements after PEEP 9 cmH2O measurements in order to avoid a further deleterious effect of gas exchange on cerebral autoregulation. The goal of this study was precisely to describe the integrative effect of respiratory mechanical, gas exchange and hemodynamic parameters on brain oxygenation, and our data do show that the effect of IRV depends on the PEEP level, and that IRV does not allow improving brain oxygenation under an inadequately low PEEP, in part due to impaired gas exchange.

2. Without measuring cardiac output it is difficult to exclude systemic changes in cardiac output on cerebral blood flow. Did the authors measure cardiac output?
R2. Unfortunately, it was not technically possible to measure cardiac output. However, we believe that the drop in both MAP and CVP at baseline suggest a drop in cardiac output. We have added this point in the study limitations section of the discussion.

3. Research about cerebral blood flow in ARDS is always complex as changes in CO2-levels and different ventilator settings have separate influence on cerebral blood flow. The authors already stated this in the limitations section. CO2-levels are different between the PEEP-levels. In my opinion this has a significant influence on brain perfusion and subsequently brain oxygenation. Please discuss.

R3. We fully agree with the reviewer and emphasize the fact that our study describes and dissect these effects in detail. Specifically, we show that despite the hypercapnia under PEEP 6 cmH2O, which resulted in an increased carotid flow, brain oxygenation was largely impaired in injured lung, due to the decrease arterial blood oxygenation. Therefore, the integrative effect resulting from changes in PaCO2, carotid flow and hypoxemia on brain oxygenation could be described. We now discuss this point on page 13.

4. Did you randomize in ARDS levels of PEEP, like the I:E setting? If not do you think that this may have an influence on results as PEEP6 was always subsequent to PEEP9?

R4. As mentioned above in R1, we did not randomize the order of PEEP administration. This is because hypoxia and hypercapnia were worse under PEEP 6 cmH2O. Because both can compromise cerebrovascular autoregulation, we did not risk a carry-over effect of PEEP 6 – on PEEP 9 cmH2O. Therefore, PEEP 9 measurements were performed before PEEP 6.


R5. We recognize that CVP is an imperfect indicator of volume status, however, it does seem to vary with hypovolemia and fluid resuscitation in rabbits (see: Chen et al. J Trauma. 2009 Mar;66(3):676-82). We have added a comment on this point in the Discussion.

Minor recommendations:
6. I would recommend to state that this study was done in animals and transferring the results to humans is not without issues.

R6. We have added a statement in the study limitation section of the Discussion.

7. The legend of figure 2 is incorrect as there are no triangles and inverted triangles in figure 2. I would recommend to delete the box in figure 2 muscle ΔHb and include symbol explanation in legend. This is also valid for figure 4.

R7. We corrected the error in the legend. Thank you. Figures were modified following the reviewer’s suggestion.