Reviewers report

Title: Effects of different flow patterns and end-inspiratory pause on oxygenation and ventilation in newborn piglets: an experimental study.

Version: 2
Date: 21 June 2014
Reviewer: Nicholas M Mohr

Reviewers report:

Thank you for the submission of this very interesting and timely report. The authors present a well-conducted laboratory study of the effects of different ventilation waveforms in an animal model of pediatric intraoperative ventilation. Overall, I find the study and reporting well done, and the question that the authors pose is one that is well suited to an animal model. They state a clear objective and the conclusion matches that stated objective. My specific comments follow:

Major Compulsory Revisions:
1. Your analysis, as described, seems to make several assumptions that are unclear.
   a. First, you have used a test for your primary outcome that relies on statistical independence (Wilcoxon). Unfortunately, for each mode, your 15 iterations are conducted on only 12 animals, so 3 of those modes are conducted on the same animal twice. There are several ways to consider this analysis (multilevel modeling, for instance). I do not expect that this change will change the significance of your results, but one should adhere rigorously to the statistical assumptions of these tests.
   b. It is not clear to me why a Bonferroni correction was applied: you have a single primary outcome. In this case, you do not approach the threshold of significance, but this should be better clarified in your results why you chose to use this strategy. Further, if you have actually applied a Bonferroni correction, it seems that p < 0.05 would no longer be accurate for each test (although this is the threshold listed in the caption of your tables).
   c. The assumptions from your sample size calculation are clear. The point estimate of your difference suggests that sample size is not a limiting factor in your ability to see a difference. One should be clear, though, that your sample size citation, strictly speaking, estimates a pO2 mean and variance in infants undergoing cardiac surgery – not healthy newborn pigs. These may be the closest data that you have to support your sample size, but one should be clear where these data originated. To be fair, your newborn pigs have much higher pO2 than the infants in the study upon which your sample size is based.
2. One significant limitation of your methodology is the inability to measure the sustained effects of one ventilatory strategy on another. While your method addresses the systematic bias of running the experiment the same way in all 12
animals, it does not allow you to comment on the sustained effects of one strategy if the effect spills over into the next 30-minute period. While your approach is more conservative, it does open you to the possibility that you have not found a difference in modes because the spillover effect contaminates all of your modes. The only way to address this would have been to avoid crossover (which would have taken more study animals), which may not be desirable. At the least, though, this should be listed as a limitation. One can also add this additional analysis to your paper (to search for crossover effects of one mode on other modes) to debunk a theory that this crossover effect has biased your results toward the null.

3. Do you have data to suggest that a 30 minute period was sufficient to see the entirety of the effects of your intervention and that the effect is gone within 20 minutes of moving to a new mode? If so, those data should be presented/cited.

Minor Essential Revisions:
1. Be careful not to refer to your study as being conducted on “infants.” It seems that this inappropriately leads the reader to think that humans were included in your study.

2. Please list the species of your study in the abstract. The authors are encouraged to review and follow the ARRIVE guidelines for reporting animal studies and in vivo experiments (Kilkenny C, Brown WJ, et al. PLoS Biol 2010).

3. It seems that those collecting data were not blinded for this study. If that is accurate, you should acknowledge this fact and comment on ways in which you have tried to ameliorate the effects of an unblinded study (e.g., standardized method for ventilator to measure pressures, collect blood gas in a standard way, selected objective outcome measures).

4. I appreciate your clear statement of objectives at the end of your Background. I would encourage the authors to articulate a hypothesis that they are testing. Also, it is unclear to me with the stated objectives why the authors went to such lengths to measure hemodynamic parameters, etc. It seems that there may have been an objective that was not stated a priori.

5. The inspiratory pause selected seems very short, and with a constant I:E ratio, it seems surprising that this pause would be expected to change lung recruitment, etc. The authors should clarify why a pause of this magnitude (10%) was selected.

6. I see no reason to represent identical data in BOTH tables and graphs. One inconsistency, though, is that the data represented seem to be the same parameters, but the point estimates in tables and graphs differ. I think presenting one is sufficient, but these differences must be reconciled.

7. Is the use of a cuffed tube realistic (presumably the amount of leakage around an uncuffed tube may differ based on mode, and on the flow rate and corresponding delta-pressure)? How about the tidal volume? Both may reflect clinical practice, but this should be clarified as the reason to use these assumptions in your experimental model.
Discretionary Revisions:

1. A significant factor in your study that is not discussed is the concept of lung injury. With increasing recognition of lung injury prevention as a goal of mechanical ventilation, it seems prudent to discuss and consider the mode of ventilation on the induction of lung injury. Your study looks at very short-term outcomes (oxygenation), so this may be only a limitation, but it may be useful to mention in your discussion and consider in future studies. It may be productive, however, to look at changes in lung compliance and oxygenation over the period of the study (you have analyzed based on the mode, but what if you look at Period #1 vs. Period #2, etc.). If there is no effect here, you can just make that comment, but it seems an important hypothesis to consider.

2. Your description (1st paragraph of Experimental Sequence) details the 15 iterations of each mode. I understand that you used each sequence exactly one time for each animal, but this is confusing because it could be interpreted as meaning that you conducted the same sequence 15 times for each animal. Please consider clarifying.

3. Consider adding a paragraph to your discussion to focus on the clinical relevance. Based on your results, do you advocate for a clinical change in practice? Can you say that your results suggest that ventilation mode doesn’t matter? What does this mean? What is the next step? Should this be taken to a human study, or do your results make this step unnecessary?

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests.