January 13, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: WJEM_AimalEVLP_01012014_unmarked.doc).

Title: Animal Models of Ex Vivo Lung Perfusion As A Platform For Transplantation Research

Author: Kevin Nelson*, Christopher Bobba*, Samir Ghadiali, PhD, Don Hayes, Jr., MD, MS, Sylvester M. Black, MD, PhD and Bryan A. Whitson, MD, PhD

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The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

Reviewer #1's Comments:1. Very well written. 2. New perspectives are needed in the Summary.

Response: to Reviewer #1:
We thank the reviewer for their insightful comments. The summary has been modified to include additional perspectives and future directions.

Reviewer #2's Comments:
General. This manuscript by Nelson and colleagues is a well-written and useful review of the literature on a timely topic that falls within the general field of experimental medicine. References are numerous and up-to-date, and cover the wide variety of applications of this technique. Specific. My single comment concerns the widespread use of the term "model" by the authors in a variety of distinct contexts, which may confuse the reader as to what is a model of what, and why it is so. While the underlying science is undisputably sound, nomenclature is important too, especially when one tries to convey important novelties to a nonspecialist reader. "Model" is very helpful term, when properly used. From the context of the manuscript, it is necessarily an experimental model of disease, or of therapy, or both. Some experimental situation may be a model for colitis, for example. This means that an experimental procedure adequately reproduces features of a naturally occurring process (colitis). Another model may reproduce artificially created conditions, such as the disturbances resulting from the use of some wide-spectrum antimicrobial agents, or from surgical resection of significant length of the small intestine. In either case, the "model" cannot be defined without a reference to the condition, or phenomenon, it is believed to mimic ("to model"). Models are needed because the study of the original conditions/phenomena is difficult, due to interference by many uncontrolled variables and/or technical difficulties that prevent adequate observation. A model allows for removal of these complicating factors but preserves the features of interest, further allowing for control of most spurious factors that will confuse interpretation. Now, a technique can provide the foundation of many valuable models, but if the technique itself does not attempt to reproduce some condition or phenomenon, it is

not a model in itself. Consider tissue culture, for example. It allows one to observe live cells outside the body, and use them to study growth, senescence, malignant transformation, etc. Tissue culture in itself is not a model for anything, however, because the conditions are very different from the situation in vivo, even in extremely complex media. Specific experimental settings, on the other hand, can be defined in tissue culture conditions, enabling us to observe in vitro phenomena that are believed to occur in vivo (for instance, tumor cell killing by immune cells) but are difficult to observe directly. Then the specific system in vitro can be said to approach reasonably the phenomenon of interest and to be an acceptable model of the latter. These considerations apply to ex vivo lung perfusion. It is a technique for keeping the lung alive and functional in a way that allows it to be examined for functional reserve and the presence of untoward factors. By careful handling ot the lungs and proper attention of a number of parameters, ex vivo lung perfusion also allows the investigator to better preserve an organ that will be transplanted later, and even recover part of the function when the organ is in suboptimal condition. Hence, the situation is analogous to that of dismantling a complex machine, such as a motor car, in order to diagnose the cause of a defect and fix it, or to conclude that it cannot be fixed. The disassembling of the complex system, with resulting isolation of the part of interest and the corresponding reduction in interfering factors, allows one to simulate (i. e., to model) a number of disturbances and to accurately measure the responses. In the case of motor cars, this is often sufficient. In the case of human organs, we are not only disturbing and recording responses, we are also learning novel things in the process. That is when the planned introduction of a specific disturbance will provide a model for a natural or iatrogenic condition. It is easy to understand that ex vivo lung perfusion is

Response: to Reviewer #2:

We feel that the term "model" is appropriate to describe both the technology and the approach to using small and large animals for ex-vivo lung perfusion in the setting of lung transplantation. We feel that EVLP is a model system in that it fits the criteria of discoveries with the platform will provide insight into the pathophysiology of human lungs, the platform allows in vivo/ex vivo approaches to study human disease where such human experimentation would not be feasible, and that the platform provides an isolated, pure system where individual variables and their effects can be studies. If the Editor would like a different term used, we would be more than happy to ablige.

Reviewer #3's Comments:

The manuscript by Nelson et al., provides very interesting discussion regarding the use of Ex-vivo lung perfusion (EVLP) as an experimental model for isolated lung research. Accordingly, EVLP allows for the lungs to be manipulated and characterized in an external environment. As a consequence, the effect of specific ventilation/perfusion variables can be studied independent of other confounding physiologic contributions. At the same time, EVLP allows for normal organ level function and real-time monitoring of pulmonary physiology and mechanics. As a result, this technique provides unique advantages over in-vivo and in-vitro models. Indeed, EVLP has great potential to increase the lung donor pool by providing a platform for improving and evaluating lungs initially thought to be inadequate. Based on this, I strongly recommend the acceptance of the manuscript after a careful revision about the Figures citations/mentions. Indeed, Figures could be better explored; in this version of the manuscript, the Figures are relatively incomprehensible.

Response to Reviewer #3:

We thank the Reviewer for their insightful review. At the Reviewer's request, we have redone the figure diagrams and have modified the figures for clarity. In addition, the superfluous images have be removed to

streamline the manuscript.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Surgical Proceedures.*

Sincerely yours,

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