**JoVE59197 - A Pre-Clinical Porcine Model of Orthotopic Heart Transplantation**

Dear Dr. Phillip Steindel,

We greatly appreciate the editorial review of our manuscript entitled “**A Pre-Clinical Porcine Model of Orthotopic Heart Transplantation**”. We have responded to each comment and made the necessary changes to the manuscript.

**Editorial comments:**  
  
**1. Protocol: Please indicate how surgery/evaluation ends, including euthanasia.**

Thank you for your suggestion. We have added this to the end of the protocol.

* 1. Experiment termination and euthanasia:
     1. Once all assessments are finished, exsanguinate the recipient animal into the venous reservoir of the CPB circuit by opening the venous line clamp. Alternatively, exsanguination can be achieved by harvesting the cardiac alograft to collect samples (i.e. myocardial biopsies).

**2. Formatted per JoVE guidelines (12 pt Calibri font, all text aligned to the left margin, spaces between each step; see attached manuscript), the protocol exceeds our 2.75 page limit for filming. Please highlight 2.75 pages or less of the Protocol (including headers 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.**

This has been modified in the manuscript.

**3. Figure 1: If possible, please use ‘mL’ (capitalized ‘L’) instead of ‘ml’ here.**

We apologize for the inconvenience. We have modified the figure.

**4. Figure 1 Legend: Please explain the ‘relationships’ a bit more here or in the Results. Please define ‘ED Volume’.**

We apologize for the inconvenience. We have modified the figure and included a brief explanation of the relationships in the results section as below:

**REPRESENTATIVE RESULTS:**

This pre-clinical model has been used successfully since 1994.5,6,8,9 Table 1 demonstrates representative results from pressure-volume relationships and echocardiographic parameters taken at baseline, and 3 hours post-transplantation in a set of 5 experiments. Although we see a decrease in myocadial contractility following transplantation, this was not statistically significant.

Figure 1 shows representative pressure-volume loops collected from one experiment at the same time-points. During “steady-state” assessments (figure 1a and b), volume-dependent parameters are recorded, such as maximum and minimum rate of developed pressure. Volume-independent parameters are obtained by intermittent occlusion of the IVC. With this, the volume of the left ventricle progressively decreases and different relationships can be calculated. In figure 1 c and d, we see the end-systolic and end-diastolic pressure-volume relationships being recorded, which represent the relationship between the end-systolic or end-diastolic pressures, respectively, with the corresponding end-diastolic volume. In figures 1 e and f, we see the recording of preload recruitable stroke work, which is the relationship between the stroke work and the corresponding edn-diastolic volume.

Finally, as seen in figure 2, various other metabolic (e.g. lactate levels and pH) and functional parameters (e.g. cardiac output) can be measured with this model to test different hypotheses.

**5. Figure 2: Please include spaces between numbers and units (e.g., “15 min”, “1 h”).**

Thank you for the suggestion. We have made the proper modifications.

**6. Table 1: What statistical test is used to produce the p-values here?**

We apologize for not including this earlier. We utilized the Wilcoxon Signed Rank Test to compare the timepoints in the table. This has been clarified in the table legend.

Once again, thank you very much for considering our manuscript for publication. We thank you for the opportunity.

Sincerely,

Dr. Roberto Vanin Pinto Ribeiro