

## Peer Review File

Article Information: <http://dx.doi.org/10.21037/tau-20-1467>

Reply 1:

*Line 106 - 250ml is standard pig bladders in our previous publications and others as shown in the following references.*

*Changes in the text:*

*To address this concern, the following text has been added to the methods (filling and dying) section: This volume was selected based on our previously published experiments and those of others (references in manuscript)."*

Comment 2: (Line 108) What was vessel pressure? How was this flow rate chosen, in relation to normal blood flow in a pig bladder? It would be ideal to also cannulate the accompanying veins to allow for a true pressurized system – although I do understand the technical complexity of this request and thus it may not be possible.

Reply 2:

*Line 108 - The flow rate of 10ml/min was chosen based on our previous publication showing that this rate was physiologic and above the threshold for ischemia. This was used to assure minimal mixing of dyes prior to colorimetric assessment.*

*Changes in the text:*

*To address this issue, the following text was added to the methods section: ".to approximate a physiologic flow rate (reference in manuscript)."*

Comment 3: (Line 108) Why was DI water chosen and not a physiological salt solution as a vehicle for the dye? This likely obliterated the endothelium throughout the vascular tree, making it difficult to know if what was seen with regard to collaterals and dye mixing was related to the inability of the vasculature to relax in response to shear stress.

Reply 3:

*Line 108 - In this initial colorimetric study, physiologic buffer was not used due to the acute nature of the imaging. Future studies aimed at the evaluation of organ function in the setting of ischemia should use physiologic solutions. This text has been added to the discussion as a limitation.*

Comment 4: (Line 149) Normalizing to yellow is incorrect in this instance. The hue of yellow is a combination of red and green light, but you are using red and green dye. This is not the same and the same color wheel does not apply. While you are analysing images (and this I can understand the use of yellow), the use of this color wheel would only work for fluorescence, where the dye being used actually gives off light. Thus, an equal mix of red and green should be used to define the hue that is a perfect mix of both, and this should be the normalization point.

Reply 4:

*Line 149 - Thank you for bringing up this important point. While we recognize the limitations of using dye (instead of substances which actually emit fluorescent light), this initial colorimetric study was simply designed to assess differences in relative vascular distribution based on bladder hemispheres.*

*Changes in the text: To acknowledge this and other limitations, we agree to change the title to reflect the*

*preliminary nature of this investigation as follows: "A Preliminary Study of Bilateral Color Mapping of Pig Bladder Vasculature Demonstrates Potential for Acute Hemi-Ischemic Events"*

Comment 5: (Line 152, and all of Results) The problem lies in how the images are acquired and the relative size of the vessels vs. tissue thickness. The surface arterioles are comparatively large, and thus will dominate in color/hue compared to the underlying tissue. Even though you are using hue and not intensity, the surface will drown out your ability to rectify any differences at the small arteriole/capillary level, which is where the collaterals seem to reside. This is particularly well-seen in Fig 4E2, as compared to Fig 1C: the surface arteries stay perfectly green, as expected but there is a definite change in hue beneath and around those arteries. Yet, Fig 4E2 doesn't seem to show this clearly. This also goes for 4J1/2.

*Reply 5:*

*Line 152/results - Again, we recognize these and other limitations and have added the phrase "A preliminary study." to the title. However, we wish to emphasize that the study was not designed to evaluate a detailed map of bladder vascular supply. Rather, this initial colorimetric investigation was designed to evaluate the overall hemispheric distribution of blood flow. Although preliminary in nature, the previously held premise that there is adequate collateral blood supply to prevent focal ischemic events may be called into question by this study.*

My minor comments are as follows (line numbers indicated as needed):  
• None.