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JoVE - Journal of Visualized Experiments

Research Topic: Cardiovascular research - Innovative animal models of cardiac remodeling: development and evaluation

Dear Editors and Reviewers:

I wish to submit the revised manuscript titled "A surgical model of heart failure with preserved ejection fraction in Tibetan minipigs." The paper was coauthored by Xiaohui Li, Weijiang Tan, Xiang Li, Shuang Zheng, Xiaoshen Zhang, Honghua Chen, Zhanhong Pan, Caiyi Zhu, and myself. I greatly appreciate the editors and reviewers for the thoughtful suggestions and insights. The manuscript has benefited from these insightful suggestions.

Thank you in advance for your time and consideration. Any comments regarding this manuscript will be greatly appreciated.

The responses to the editors and reviewers are attached.

Sincerely yours,

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Responses to the editors and reviewers

Editorial comments:
Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

Response: We have edited this manuscript.

2. Please reword the following lines to avoid previously published work: 24-25, 37-38, 164-167, 177-179.

Response: We have revised these sentences.

3. Please provide an email address for each author.

Response: The email addresses are listed below.
  Xiaohui Li, email: lixh1989@jnu.edu.cn
  Weijiang Tan, email: twj@gdlami.com
  Xiang Li, email: lx@gdlami.com
  Shuang Zheng, email: zhengshuang@gdlami.com
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  Zhanhong Pan, email: zhanhongp@qq.com
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  Caiyi Zhu, email: 2561715295@qq.com
  Feng Hua Yang, email: fenghua.yang@gdlami.com

4. Please provide a Summary before the Abstract to clearly describe the protocol and its applications in complete sentences between 10-50 words: "The present protocol describes …"

Response: The present protocol describes a step-by-step protocol to establish a minipig model of heart failure with preserved ejection fraction using descending aortic constriction, as well as the approaches to evaluate the phenotypes of this model.

5. Please ensure that abbreviations are defined at first usage.

Response: We have checked.

6. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials.

For example: Shenxin®

Response: Thanks. We have revised these.

7. Please note that your protocol will be used to generate the script for the video and must contain everything that you would like shown in the video. Please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.

Response: Thanks for the information.

8. Please add more details to your protocol steps:

Step 1.4: Please ensure that the details of all the materials/reagents/equipment mentioned are included in the Table of Materials.

Step 2.3: Please include the details of the echocardiography device in the Table of Materials. Also, please mention how much blood sample was collected.

Step 2.5: Please mention how long the animals were kept under the said conditions.

Step 3.13: Is the benzylpenicillin powder the same as the benzylpenicillin injection mentioned in the Table of Materials? If not, then please include the details.

Step 4.2: Please include the details of flunixin meglumine in the Table of Materials.

Response:
Step 1.4: All have been checked.
Step 2.3: We have revised the description of this equipment.
Step 2.5: We have revised the sentence as follows: "2.5 Initiate the ventilation at tidal volume 8 ml/kg, 30 breath/minute and maintain the animals with 1.5–2.5% isoflurane during the surgical procedure"
Step 3.13: They are the same benzylpenicillin injection. We have revised that in the context and that table.
Step 4.2. We have deleted flunixin meglumine.

9. Please include one line space between the protocol steps and highlight 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. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.

Response: Yes. We have highlighted the key steps by following the instruction.

10. Please ensure that the highlighted steps form a cohesive narrative with a logical flow from one highlighted step to the next and also is in-line with the Title of the manuscript. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in the imperative tense. However, the NOTEs cannot be filmed, so please do not highlight them.

Response: Thanks for the information.

11. As we are a methods journal, please ensure that the Discussion cover the following in detail in 3-6 paragraphs with citations:
a) Critical steps within the protocol
b) Any modifications and troubleshooting of the technique
c) Any limitations of the technique
d) significance with respect to existing methods
e) Any future applications of the technique

Response: Thanks. In the discussion section, we added one more paragraph to indicate the limitation of this technique.

12. Please ensure that all the Figures (including the sub-panels) are referenced in the manuscript text in sequential order.

Response: Thanks for the information.

13. Figure 3I/5B: Please include the description in the figure legend.

Response: We have added the description to Figure 3I, and added "(B)" following the description in Figure 5.

14. Figure 4: For 4A-please include scale bars. 4B-E-please include the x-axis descriptions.

Response: We have added scale bars indicating the scanning depth for B-mode images and the recording time for M-mode images in Figure 4A. Descriptions of x-axis were added to Figure 4B.

15. Figure 7: Please mention in the Figure legend what the arrows in yellow and the asterisks marks indicate.

Response: We have added the description for these symbols.

16. Please do not abbreviate the journal names in the References.

Response: We have changed the reference style.

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Reviewers' comments:

Reviewer #1:

Manuscript Summary

Xiaohui Li et. al. described a model of heart failure with preserved ejection fraction in Tibetan minipigs. They presented the protocol step-by-step with essential details. The paper writting is well organized.

Major Concerns:
I would like the authors to provide more specific application prospects of this model in the research field and discuss the limitation if it has.

Response: We have discussed the limitation of this protocol.

Minor Concerns:
The charts in Fig 4 missed the title of x-axis.

Response: The description of x-axis was added to Figure 4.

Reviewer #2:

Manuscript Summary:

Authors described in detail the surgical procedure of establish a large animal HFpEF model using descending aortic constriction (DAC) in Tibetan minipigs. Cardiac function, heart injury including fibrosis, inflammation, and cardiomyocyte hypertrophy, heart failure markers were fully examined. The model of HFpEF is helpful to elucidate the molecular mechanisms or preclinical findings. The manuscript was well written,

Major Concerns:

1. NT-proBNP is usually detected as a heart failure marker in patients. It is of great significance if authors provide the index.

Response: Thanks. We agree that various biomarkers are used in diagnosing heart failure. We have shown expression of ANP and BNP in the DAC hearts were significantly higher than that in the sham hearts in a previously published paper (Ref: Tan, et al., Front. Cardiovasc. Med., 2021, doi: 10.3389/fcvm.2021.677727).. Here, changes of serum cTnI at different timepoints were added to characterize the physiological condition of the HFpEF minipigs.

2. Advice authors to provide clear evidence of cardiac fibrosis using masson staining.

Response: We have previously reported that the fibrotic area, stained by Sirius Red staining, in the DAC group was significantly greater than that in the sham group. (Ref: Tan, et al., Front. Cardiovasc. Med., 2021, doi: 10.3389/fcvm.2021.677727)

Minor Concerns:

In fig 4, B-E, It is more clear that one curve represents sham group and the other curve represents DAC group. The every point indicates the value: mean ±SED

Response: Thanks. We have calculated the SEMs of each group at different timepoints and showed the graph in a summary data style of GraphPad prism 8.

In fig 5. N=3? Statistical method should be described.

Response: The statistical method was added to the legends in Figure 4 and Figure 5.