Author's response to reviews

Title: Possible mechanism underlying the effect of Heshouwuyin, a tonifying kidney herb, on sperm quality of aging rats

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Author's response to reviews: see over
Dear Dr. Tom,

With this letter we submitted our revised manuscript. The following is our point by point answer to the questions raised from reviewers.

**Reviewer #1**

Q1 The results are badly written, quite difficult to be comprehended. Figures are very badly managed, in particular graphics. The superscript letter are useful to determine the difference between the treatments, but in this case they have been misused.

**Answer:** The results are partially re-organized and are adjusted, some description in the result has been deleted to make it more user-friendly. Images are re-organized and are adjusted, and the resolution of the image are increased to make it more clear. The superscript letters of figure are modified. Differences between groups are compared using one-way analysis of variance. Intergroup comparison is analyzed using SNK-q test. The statistical significance is labeled as *P < 0.05* and **P < 0.01** where ‘a’ compared with normal control group, ‘b’ compared with aging group and ‘c’ compared with Shouwu pill group.

Q2 In statistical analysis, the new data from cells (immunofluorescence for StAR and P450) count should be analyzed with a model with Poisson errors! Alternatively, if the Authors would express these data as percentage of positive cells on the total number of cells counted (that seems to be more appropriate), they can use one way ANOVA.

**Answer:** These data are presented as percentage of positive cells on the total number of cells counted, showed in Figure 10(Ⅱ) and Figure 11(Ⅱ). The data are analyzed with one way ANOVA.
Q3 In addition the paragraph on testicular tissue evaluation is not acceptable. They cannot describe what they see without any measurements; differences must be scientifically expressed with numbers derived from measurements.

The approach on seminiferous tubules area is good, but no mention of the specific technique used to calculate this area is present in the M and M section.

**Answer:** The description of testicular morphology of has been removed in the revised manuscript. The testis are evaluated by calculating the testis parameter, measuring the average area of the seminiferous tubules, counting the number of seminiferous epithelium, as showed in Figure1 and Figure2. The areas of seminiferous tubes were measured through planimetry with image analysis software (Image Pro Plus 6.0). Related methods have been added to the Materials and Methods section, in the paragraph “Observation of the areas of seminiferous tubes and layers of seminiferous epithelium”.

Q4. The first 4 lines of the Results paragraph concerning “Testicular parameter changes” belong to Materials and Methods. These are not results. These are methods!

**Answer:** This part has been moved to the Materials and Methods section.

**Reviewer #2**

**No any question need to be answered.**

Thanks so much for your endeavors on this manuscript!

Best wishes,

Zhenshan Wang