Reviewer's report

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

Version: 3 Date: 4 December 2013

Reviewer: Diego Bucci

Reviewer's report:

General comments
The study is quite well designed; anyway there are some important concerns that need to be addressed to render the article acceptable for publication. In particular the structure is poorly organized, materials and methods should be improved, the results section should be thoroughly revised, as well as the discussion, that is too speculative.

Specific comments
Abstract and introduction are quite well written and furnish the needed information.

Materials and methods
There is no mention to the methods adopted to obtain the histochemical images of testicular tissues.

The “Results” paragraph entitled “Quantitative analysis of experimental animals” should be put in this section.

The “Results” paragraph entitled “Leydig cell aging models” should be put in this section.

An explanation of the technique used to determine the relative band intensity of western blot analysis should be provided.

Statistical analysis. Did the authors perform a normality test to assess the normal distribution of their data? And did they perform an homoscedasticity test? These tests are necessary before proceeding with an ANOVA test. In case of non-normality or heteroscedasticity the data should be analyzed using a non-parametric test.

Results
Morphological observation of testicular tissue. This paragraph is not acceptable. The Authors do not furnish any data, they only assert that there are no changes. They should provide count data on cellular populations.

StAR and P450scc in Leydig cells. The Authors should provide the data from cells subpopulations count. The Authors declare they studied mRNA expression.
No mention of the technique used for this purpose in the M and M section.

Discussion

This section is redundant. The Authors should shorten it and comment their data instead of speculate on other matters. I would suggest to delete lines from “The importance of reproductive health …..” to “… will play an important role in this point”, as well as lines from “About 99%....” to “…testosterone synthesis [35]”.

Figure and captions

Figure 1 is not necessary.

Figure 2, 7, 8, 9, 10 and 13. Instead of * and # use letters as superscript to better delineate the differences.

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, and I have assessed the statistics in my report.