

# Peer Review File

Article Information: <https://dx.doi.org/10.21037/tau-21-789>

## Review Comments:

This manuscript proposes a new mechanism in spermiation involving ASB17. These are my concerns and suggestions:

1. Page 3 (Abstract): The abbreviations of “ASB17” and “ESPN” should be described in full in the first place.

Response: Thanks for this suggestion. In the new revision, we have added the abbreviations of “ASB17” and “ESPN” in the text when first mentioned. (see, Page 3, line 46 and 50)

2. Page 3, line 46: Please mention “...approach epididymal sperm parameters...”!

Response: Thanks for this suggestion. In the new revision, we revised the sentence as: “Epididymal sperm parameters were assessed by a computer-assisted sperm analysis (CASA) system.....”. (see, Page 3, line 47).

3. Page 8 (Methods) line 167: How many mice per group?

Response: We are sorry for this confusion. Actually, as we mentioned in figure legends (see, Page 20, line 481-483), sample number was 3 for each group.

4. Page 8, line 176: Which “sections”? “Testicular sections”? Please mention it!

Response: Thank you for this comment. In the new revision, we revised the sentence as: “Testicular sections were deparaffinized and rehydrated.....”. (see, Page 8, line 177)

5. Page 9, line 192: Which “sections”? “Testicular sections”? Please mention it!

Response: Thank you for this comment. In the new revision, we revised the sentence as: “In brief, paraffin testicular sections were deparaffinized.....”. (see, Page 9, line 193)

6. Page 11, line 240: Please mention “Epididymal sperm analyses” instead of “Semen Analyses!

Response: Thanks for this suggestion. In the new revision, we revised the sentence as: “epididymal sperm analysis based on CASA.....”. (see, Page 11, line 241).

7. Page 11, line 242: There is nothing in the method section about “sperm morphology evaluation” (better term instead of “sperm deformity”). Please describe it in the Method section!

Response: Thanks for this suggestion. In the new revision, we revised the sentence as: “sperm morphology evaluation was also comparable.....”. (see, Page 11, line 243).

In addition, we also follow the reviewer’s advice and add “sperm morphology evaluation” into “Methods” section. (see, Page8, line170-171).

8. Page 20, Figure 1B (Asb 17 +/-): In the “Merge” panel, which cells are positive in the right lower corner? Spermatogonia? Please provide the information in Figure legend (1B)!

Response: Thanks for the good question! We follow the reviewer’s advice and add “In *Asb17*<sup>+/+</sup> testes, ASB17 is mainly distributed around spermatid heads (asterisks). Meanwhile, in several spermatogonia, ASB17 can also be labeled (arrows).” into “figure legends” section. (see, Page 20, line 472-474).

9. Page 22, Figure 3A: did you quantify the number of tubules with “retained mature spermatozoa” per mice group, or is this the gross observation? Please clarify it in the manuscript!

Response: Thank you for the good question! In our new revision, we quantify the number of tubules with “retained mature spermatozoa” per mice group and provide the quantitative data in Figure S3. (see Supplementary Material, Page 5).

10. Page 27, Supp Table 1: Please add a column to explain each antibody is a marker of what!

Response: Thanks for this suggestion. In the new revision, we have added the explanation. (see, Supplementary Material, Page 1)

11. Page 27, Supp Table 1: Please add Antibody information for both “PNA” and “F-actin”!

Response: Thanks for this suggestion. In the new revision, we have added the explanation. (see, Supplementary Material, Page 1)

12. Page 28, supp Table 2: There is no information or explanation about this table in the manuscript!

Response: We are sorry for this confusion. Actually, as we mentioned in Results (see, Page 13, line 279-281), “the key residues for protein-protein interactions (PPIs) are provided in Table S2.”

13. Page 29, Supp Figure 1A: is this data based on “Microarray analyses,” “RNA Seq,” or something else?

Response: Thanks for this question. In the new revision, we have added the explanation. (see, Supplementary Material, Page 3). “The data are acquired from the BioGPS database based on microarray analysis (<http://biogps.org/>).”