We would like to thank you for your careful and thorough reviewing our submitted manuscript. We sincerely appreciate your thoughtful comments and constructive suggestions, which help improve the quality of this manuscript.

All changes are made in the revised manuscript according to your scholastic suggestions. The questions raised by you are sincerely and respectfully answered in the revised manuscript and in this response letter.

**Your Concern:**  
1. I did not see anything about statistical analysis. How did you analysis your data? Did you repeat your experiment? How many replicates did you consider for your test? What did figure (e.g. 33.20±1.4) represent?

Our Response:

All statistical analyses were performed with the Statistical Analysis System “R” software package. Data for the absorbance value, live and dead sperm, and hole number were subjected to one-way ANOVA, followed by the Tukey-Kramer test. Values are presented as the mean ± SEM (n = 5) of two independent experimental replicates, and five replicates were considered for the test.

**Your Concern:**  
2. Usually we have a relatively high variability regarding sperm penetration holes. But you have low Standard deviation or Standard error in the current study. Could you explain more about data collection method?

Our Response:

We had collected F1 follicular Inner perivitelline layer (IPVL) from laying hens for sperm penetration test. As it is well known that germinal disc region (GDR) possesses 15-20-fold more sperm-binding ligands made of glycoprotein than non-germinal disc region (NGDR), thereby attracting more sperm to bind to and penetrate into the GD region than the NGD region. To avoid this type of variability between GD and NGD for penetration holes, the present method involved pre-removal of GDR making hole in the follicle and collection of entire NGDR only in intact form to allow making many replicate pieces.