Author’s response to reviews

Title: Quantitative proteomic characterization of human sperm cryopreservation: using dataindependent acquisition (DIA) mass spectrometry

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Version: 2 Date: 26 Aug 2019

Author’s response to reviews:

Many thanks to the reviewers and editors for their comments. Your opinions have been very helpful for the presentation of our research results and the publication of the article. We have revised the terms "up regulated" and "down regulated", and replaced them with "increase", “decrease ” instead.(Page 2, Line 7)

Additionally, to more clearly present the relevant content, we have employed the editing service provided by Elsevier to polish the language of the article.

Background:
Page 2, Line 25 - This sentence is awkwardly worded.
We corrected this error. (Page 2, line 23)

Page 3, Lines 7 & 9 - The authors' use of both spermatozoa and sperm is inconsistent. Either is correct but usage should be consistent throughout a manuscript including its tables and figures.

We use the term "sperm" in a unified way.

Page 3, Line 10 - It should be proteins not protein.

We corrected this error. (Page 3, Line 9)

Methods:

Page 3, Line 30 - It should be "were" was because 9 cryopreserved and fresh samples were used. The term, was, is singular.

We corrected this error. (Page 3, Line 28)

Page 4, Line 1 - It should be: After complete liquefaction, each semen sample was transferred.

We corrected this error. (Page 3, Line 33)

Page 4, Line 2 - It should be 37°C not just 37.

We corrected this error. (Page 3, Line 34)

Page 4, Lines 2 & 3 - This sentence is awkwardly worded. Suggest: Glycerol-egg-yolk-citrate (GEYC) was used as cryoprotectant. A serious question that was not addressed: Was the control sample also diluted with GEYC? Another problem is how was the possible contamination of the human sperm with egg yolk proteins addressed. Some of these avian proteins likely were bound to the sperm. How did the authors deal with the presence of avian as well as human proteins in samples?

Our aim was to compare protein differences between fresh sperm and sperm after cryopreservation. Both the cryopreservation process and the cryoprotectant are necessary for sperm freezing and are also important factors in sperm damage. So Therefore, the control group was not diluted with GEYC.

Methods for removing avian proteins: First, as in previous studies[12], by centrifugation and washing, elute proteins attached to the surface of the sperm, such as egg yolk, to the surface of
the sperm were eluted by centrifugation and washing. Secondly, in proteomic analysis, the database was derived from the human protein database. And, in the protein verification experiment, the primary antibody is anti-human antibody.

Page 4, Line 11 - It should be were not are as research data are presented in the past tense. We corrected this error (Page 4, Line 7).

Page 4, Line 12 - What is meant by small portion? Was is one straw?

According to WHO standards, 10 microliter specimens were selected for sperm quality analysis (Page 4, Line 11).

Page 4, Line 13 - It should be sperm quality assessments not semen quality assessments. We corrected this error (Page 4, Line 12).

Page 4, Line 16 - It should be noted how the fresh samples were handled during the time that the cryopreserved samples were being processed.

The protein sample is frozen at -80 degrees. Later, proteomic analysis experiments were carried out (Page 4, Line 22).

Page 4, Line 18 - It should be: Five hundred microliters not 500 µl (Also, it µL not µl). When one starts a sentence with a number it should be written out. We corrected this error. (Page 4, Line 17)

Page 4, Line 20 - What sonication instrument was used and what was the settings?

Then the samples were sonicated (Sonics, USA) at 20 joules for 2 s × 10 at intervals of 15 s, (Page 4, Line 20)

Page 4, Lines 29 & 30 - This sentence contains some word usage problems. It should be: One hundred microliters of urea buffer containing 50 mM iodoacetamide was added to the ultrafiltration tube and it was then incubated for 20 min in the dark. (The terms, add and incubate, are in the present tense not the past tense). We corrected this error. (Page 4, Line 31-32)
The abbreviations, FWHM and AGC, should be defined for readers.


Page 6, Line 33 - This sentence contains some word usage problems. It should be three washes not three washed;

We corrected this error. (Page 7, Line 2)

Page 6, Line 34 - The room temperature should be provided. (18-22°C?)

In the text, we have marked the room temperature: at room temperature (18-22°C). (Page 7, Line 4)

Results:

Page 7, Line 22 - As noted previously the authors' use of "down regulated and up regulated" as well as their use of deregulated proteins remain a problem.

We corrected this error. (Page 7, Line 27)

Discussion: Page 9, Lines 6, 8, 33 & 34 - The authors' use of both spermatozoa and sperm is inconsistent.

We corrected this error.

Conclusions: Page 10, Line 26 & 27 - As noted previously the authors' use of "down regulated and up regulated" remain a problem.

We corrected this error. (Page 11, Line 2)

References: Some references are abbreviated, but some are not. Formatting needs to be consistent and according to subjected guidelines.

We used endnote software to edit the references again.

Tables:

Table 1 - The title should include that the sperm are human. Also, the term, raw, has several meanings. Perhaps, unfrozen. The fact that these sperm motility data were obtained using a Computer Aided Sperm Analysis (CASA) system should be included.
Table 1 Characteristics of motility of fresh and post-thaw human sperm (n=14) (Mean±SD)

Table 2 - The abbreviation, KEGG, needs to be defined so that the table can stand alone. One should be able to understand what is being presented without going back to the text to get specific information.

Table 2 The detailed information of the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment results.

Figures:

Figure 1 - The title needs to include that these classification data are for human sperm

We corrected this error.(Figure 1) Fig. 1 Gene Ontology analysis classification and the enrichment result of differentially expressed proteins in the human freeze-thaw samples compared to the fresh group, in terms of Biological Process, Cell Component and Molecular Function.

Figure 2 - The abbreviation, KEGG, needs to be defined so that the figure can stand alone.

We corrected this error.(Figure 2)

Overall: This revised manuscript, which presents a quantitative proteomic characterization of the differences between unfrozen and cryopreserved human sperm using DIA mass spectrometry, contains some very interesting information. However, it also has numerous language usage errors that need correction. Also, several items relative to the methods utilized in the processing need clarification including how the unfrozen sperm were handled while the other aliquots were cryopreserved. The manuscript needs careful review by someone very familiar with the English-based scientific literature. This reviewer is aware that writing a scientific article in another language is extremely difficult even with good Comprehension of that language."

Additionally, to more clearly present the relevant content, we have employed the editing service provided by Elsevier to polish the language of the article. In the attachment, we uploaded the language polishing certificate.