Reviewer’s report

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

Version: 1 Date: 11 Jul 2019

Reviewer: Reviewer 2

Reviewer's report:

"PEER REVIEWER ASSESSMENTS:

OBJECTIVE - Full research articles: is there a clear objective that addresses one or several testable research questions? (Brief or other article types: is there a clear objective?)

Yes - there is a clear objective

DESIGN - Is the current approach (including controls and analysis protocols) appropriate for the objective?

No - there are minor issues

EXECUTION - Are the experiments and analyses performed with sufficient technical rigor to allow confidence in the results?

Yes - experiments and analyses were performed appropriately

STATISTICS - Is the use of statistics in the manuscript appropriate?

Yes - appropriate statistical analyses have been used in the study

INTERPRETATION - Is the current interpretation/discussion of the results reasonable and not overstated?

No - there are minor issues

OVERALL MANUSCRIPT POTENTIAL - Has the author addressed your concerns sufficiently for you to now recommend the work as a technically sound contribution? If not, can further revisions be made to make the work technically sound?"
Probably - with minor revisions

PEER REVIEWER COMMENTS:

GENERAL COMMENTS: This revised manuscript presents a quantitative proteomic characterization of the differences between unfrozen and cryopreserved human sperm using data-independent acquisition (DIA) mass spectrometry. The authors have not properly addressed the concerns of Reviewer 2 relative to the use of "up-regulation" and "down-regulation. This manuscript does present some very interesting information but also contains numerous language usage errors that need correction. Several items relative to the methods utilized need clarification in addition. Other items that need to be addressed are noted below.

REQUESTED REVISIONS:

The problem with up and down regulation need to be resolved.

Abstract:

The authors' use of "down regulated and up regulated" as well as their use of deregulated proteins remain a problem. These terms have the connotation in the English language that the biosynthesis of these proteins was affected by down or up regulation of gene expression originating from the DNA such that RNA levels were modified such that either RNA synthesis was reduced and fewer proteins were made (down regulated) or that new proteins were synthesized (up regulated). The authors have not properly addressed the concerns of Reviewer 2 relative to the use of "up-regulation" and "down-regulation. Although some protein biosynthesis occurs in sperm it is a minor component and the interaction between seminal plasma proteins and sperm are another potential confusing factor. It is well established that even epididymal proteins, and even egg yolk proteins, bind to sperm and that some remain very strongly bound to the gametes. Also, there is a problem with potential proteolysis in the samples during the processing. Sperm contain many proteolytic enzymes that degrade proteins and have affected the resultant data presented in this manuscript.

Background:

Page 2, Line 25 - This sentence is awkwardly worded.

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.

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

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

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

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

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?

Page 4, Line 11 - It should be were not are as research data are presented in the past tense.

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

Page 4, Line 13 - It should be sperm quality assessments not semen quality assessments.

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

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.

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

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).

Page 6, Lines 3-5 - 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;

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

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.

Discussion:

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

Conclusions:

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

References:

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

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 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.

Figures:

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

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

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.

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As a minimum standard, please include a few sentences that outline what you think are the authors’ hypothesis/objectives, their main results, and the conclusions drawn. Your report should constructively instruct authors on how they can strengthen their paper to the point where it may be acceptable for publication, or provide detailed reasons as to why the manuscript does not fulfill our criteria for consideration. Please supply appropriate evidence using examples from the manuscript to substantiate your comments. Please break your comments into two bulleted or numbered sections: major and minor comments.

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