Response Reviewer 1

Response to the Editor and Reviewers' comments

Ms. Ref. No.: cells-1591705

Title: „The role of mitochondria in human fertility and early embryo development: what can we learn for clinical application of assessing and improving mitochondrial DNA?"

Journal: Cells

General comment:

We would like to thank the Co-Editors-in-Chief and Reviewers for their comments and insights. We appreciate your input and have revised the manuscript in line with your suggestions. We hope that the revised version will meet with your approval. Below are our responses to all the Reviewers' comments.

All changes are marked up using the "Track Changes" function.

Answers to the Reviewer's Comments:

Reviewer's Comment: In the abstract the authors write 'As mitochondrial DNA is exclusively maternally inherited, oocyte's mitochondrial DNA level is crucial to provide sufficient ATP content for the developing embryo until the blastocyst stage of development.'. Later in the manuscript the authors report cases of paternal mtDNA transmission, though, this is not a general mechanism. The authors should therefore tone down their statement by e.g. adding the word 'generally'.

Authors' Response: Thank you for this suggestion. We have added the word "generally" in mentioned sentence of the abstract.

Response Reviewer 1

Reviewer's Comment: In the introduction, line 35, the authors describe the length of mtDNA with 16.6 kb. However, this only refers to human or primate mtDNA, as mouse mtDNA has e.g. a length of 16.3 kb. Please clarify.

Authors' Response: Thank you very much for this remark. In terms of the length of mtDNA we had in mind human mtDNA, thus we have clarified it in the introduction.

Reviewer's Comment: The paragraph starting with line 51 needs to be rewritten as it contains misleading information. The authors should consider obtaining the information from original literature instead of reviews reporting wrong statements themselves. E.g. in lines 58, 59, and 60 they write: '16,6 kbp MtDNA controls the synthesis of about 67 proteins, including 13 of the OXPHOS, while the rest of the bacterial genes were transferred to the nuclear genome [3,7,8].'. This is not true and was already misinterpreted in the cited review paper. The mentioned 67 proteins are encoded by the mitochondrion with the largest coding repertoire, that of *Reclinomonas americana*. Human mtDNA (which has a length of ~16.6 kb) only encodes 13 proteins.

Authors' Response: After careful consideration of the Reviewer's comment we agree that above-mentioned statement was misinterpreted in the cited paper. Therefore we have corrected the sentence and removed this reference from the manuscript.

Reviewer's Comment: In lines 142, 143, and 144 the authors state 'Reaching the threshold of mutation frequency leads to mitochondrial dysfunction and thus, severely affected offspring (Figure 1) [30,31].'. Not every mutation/variant in mtDNA leads to mitochondrial dysfunction. Among other, there are e.g. variants defining the different mtDNA haplogroups which are not associated with any disease or synonymous mutations. To avoid misunderstanding, such information should be added to the text.

Response Reviewer 1

Authors' Response: We agree with the Reviewer that not all of the mtDNA variants are disease-causing. Clearly, the phenotypic threshold regards only the disease-causing ones. Thus, we have clarified it in the mentioned sentence and included the information suggested by the Reviewer with proper reference.

Reviewer's Comment: Regarding the bottleneck theory (paragraph starting with line 159): The reduction in mtDNA number in PGCs is not the only factor that can contribute to the observed germline mtDNA bottleneck, as e.g. discussed in a review by Stewart and Chinnery (Nature, 2015, https://www.nature.com/articles/nrg3966.pdf). According to shifts in frequency of heteroplasmies from one generation to the next, the size is actually much smaller that the ~200 mtDNA copies in PGCs. To avoid wrong conclusion, the authors should include such information.

Authors' Response: Thank you very much for this remark. After careful consideration of the publication suggested by the Reviewer, we have made necessary corrections in the text.

Reviewer's Comment: In lines 249 and 250 the authors write: 'As the mitochondrial genome has only exons and no introns, any point mutation or deletion could disrupt cellular respiration [58].' This sentence is misleading as someone could think that mtDNA only consists of exons, while there is also non-coding DNA (D-loop). Please clarify.

Authors' Response: Thank you for this comment. Indeed, this sentence may be misleading, thus we decided to clarified this statement in the manuscript.

Reviewer's Comment: In the legend of Table 1, please explain what N refers to.

Authors' Response: N refers to sample size. We have added this explanation in the legend of Table 1.

Response Reviewer 1

Reviewer's Comment: There are still several formatting issues in the text such as double-spaces or reduced font size of some paragraphs.

Authors' Response: We have edited the manuscript to correct above-mentioned formatting issues.