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MASTER THESIS REVIEW

Student name:	**Valeriy Kutsyna, Bc.**
Thesis title:	Assaying mitochondrial cell number, morphology and respiration status in the emerging three lineages of the preimplantation stage mouse embryo
Affiliation:	Faculty of Science, University of South Bohemia, České Budějovice, Czech republic.
Supervisor:	prof. Alexander W. Bruce, Ph.D
Reviewer:	prof. Marta Czernik, PhD; D.Sc
Reviewer's Affiliation:	Experimental Embryology Laboratory Department of Veterinary Medicine University of Teramo, Teramo, Italy

In the Master Thesis submitted for evaluation, Bc. Valeriy Kutsyna presented and discussed mitochondrial morphology and function at the late mouse blastocyst paying special attention to the differences between inner cell mass (ICM) and trophectoderm (TE) lineages. Additionally, he has explored the role of p38-MAPK activity in mitochondria maturation dynamics.

Many articles highlight the importance of mitochondrial function in regulating pre- and post-implantation development and demonstrate that correct mitochondrial function is required for normal development. Despite the developmental plasticity of the embryo, impaired mitochondrial function affects subsequent foetal and placental growth. Despite many years of investigations into mitochondria in preimplantation embryos mitochondrial morphology and function in relation to the emerging three blastocyst stage lineages (i.e.

trophectoderm/TE, primitive endoderm/PrE and epiblast/EPI) is not fully investigated and understood. Therefore, the tasks set by the Master student are considered well justified.

Bc. Valeriy Kutsyna, firstly characterised morphologically and physiologically mitochondria in the preimplantation embryos developing tissues of late mouse blastocyst and then he investigated whether p38-MAPK plays a role in mitochondria development, differentiation and lineage-specific segregation.

Referring in more detail to the experiments described in the Master thesis, it can be said that they were planned correctly, well performed and based on original hypotheses. Moreover, the thesis is written quite well in terms of language and logically organised into chapters and subchapters.

The **Introduction** is a comprehensive study and contains a clear and concise overview of the current knowledge on the pre-implantation development of the mouse embryo with extraction, the emergence of the first cell lines, role of p38-MAPK in the pre-implantation embryo development as well as mitochondrial structure and function in the mouse embryos.

The aim of the work has been concisely and clearly presented and divided into 2 main parts which goals were to investigate the structural organisation of preimplantation mitochondria networks in the mouse model.

Unfortunately, the **Materials and Methods** chapter is not neatly written. It is hard to follow experimental design and understand the major steps. Since the author did not present a comprehensive scheme (in the Material and Methods section) of the research carried out excluding all threads and procedures, understanding the set of experiments required me to analyse this chapter a few times. Moreover, the number of the embryos used for each experiment, experimental replicates, antibody used for immunofluorescence staining etc. were not included in the main text but were performed as supplemental tables. This creates little confusion and makes the Material and Methods chapter hard to follow.

Whereas, the Master's student properly fulfilled the planned tasks and correctly present them in the **Results**.This chapter is easy to read, firstly because of the logical organisation of the material into chapters and secondly because of the correct illustration by the included charts, photos, and diagrams, which are clear and allow the reader to evaluate the results for himself.

I would like to point out the most important (in my opinion) results obtained in this Master thesis:

1) It has been shown, for the first time, that p38-MAPK influences trophectoderm (TE) as the relationship between TE cell mitochondrial polarisation via p38-MAPK regulation. This observation opens a new strategy for the study of novel regulatory mechanisms in mouse embryo development;

2) The inhibition of p38-MAPK affects the transcriptional activity of electron transport chain (ETC) complex genes at the E4.5 mouse embryos;
3) Inhibition of p38-MAPK activity during mouse blastocyst maturation may cause the impaired mitochondrial fission, showing as increased and consolidated mitochondrial network;
4) p38-MAPKi during the blastocyst maturation stages of development (E3.5 - E.4.5) leads to a down-regulation of several mRNA transcripts responsible for protein production in electron transport chain (ETC) complexes I, III, IV, and V which may suggests that p38-MAPK signalling may have a broader mitochondrial regulatory role in OXPHOS and ATP production activity during mouse blastocyst development.

The **Discussion** chapter, in my opinion, is the best part of the thesis, the results are well discussed and analysed in relation to available literature. The Master student showed a wide knowledge of the literature in the topic. His ability to concentrate on the subject deserves attention. The only criticism here may be the lack of the short section of the Conclusions to underline the major results. The **Literature** is relevant and well chosen.

Detailed comments:

1) Many articles confirm that *in vitro* culture conditions might affect the development, morphology and function of mitochondria of mouse (and not only) embryos. In the presented work, 2-cell stage (E1.5) embryos were collected from oviducts and *in vitro* cultured till E3.5/4.5 stage embryos. Two/three days of *in vitro* culture may affect the mitochondrial functionality and dynamics. I would suggest having additional control which is naturally mated embryos flushed at day E3.5/4.5 stage embryos to verify eventual *in vitro* culture effect.

2) The reference on which the MAPK selective inhibitor (SB220025) concentration was selected should be given.

3) Further experiments involving combining a stable mitochondrial label with other fluorescent biosensors for mitochondrial redox state, ROS analysis would be worthy to study. It would provide valuable insights into mitochondrial structure-function relationships in preimplantation embryo development.

4) Morphology of the mitochondria, maintained by a balance between their fission and fusion, is critical for proper cellular/embryo function. Numerous studies have suggested that an imbalance in mitochondrial fusion/fission might lead to lower blastocysts quality. Interesting, preliminary result presented here, showed that inhibition of p38-MAPK activity during mouse blastocyst maturation may cause of impaired mitochondrial fission. This observation should be confirmed also by additional analysis.

5) Proposed method for the analysis of the mitochondria morphology as well as 3D reconstruction is based on the extremely high quality of the three-dimensional (3D) confocal scans as well as complex photo editing. I am concerned that a small deviation or delicate inaccuracy in the procedure as well as photos quality and preparation (editing) may affect the final results.

Final evaluation: Over all, the Master thesis written and discussed by Bc. Valeriy Kutsyna is of high scientific merit, and deserves a grade A (excellent).

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