SLOVAK UNIVERSITY OF AGRICULTURE IN NITRA
FACULTY OF BIOTECHNOLOGY
AND FOOD SCIENCES

Department of Biochemistry and Biotechnology

Reviewer´s comments on PhD thesis

The author of the PhD thesis: Mgr. Miriama Štiavnická
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The title of PhD thesis:
Epigenetic modifications of the sperm and the application in clinical practice of human assisted reproduction therapy

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Oocytes and spermatozoa of a good quality are the main prerequisites for the successful fertilization and embryo development. The development and differentiation of gametes is quite sensitive to the lifestyle of individuals that has the potential to change epigenetic information in gametes and thus influence the embryo fate. Nowadays, an increasing number of couples are forced to use the services of assisted reproduction centres due to their infertility. However, with the increasing use of assisted reproductive therapy (ART), also the incidence of genetic disorders rises as the natural selection barriers are bypassing. Therefore, the quality of gametes in connection with their epigenetic changes are crucial for the improving of ART outcomes. In this PhD work author focused on the promising epigenetic markers of gametes quality that are used for ART therapy.

The thesis meets all requirements for such type of documents. It is clearly written and properly divided into individual chapters. In the literature review, written on 28 pages, PhD student analyses the development of gametes as well as the sperm epigenome and the epigenetic changes during spermatogenesis. A more than 100 cited references were used in this thesis. Majority of them are foreign and within the last 5 to 10 years.

The presented thesis has several aims that are clearly formulated as follows: 1st to detect H3K4me2 in sperm with different quality using flow cytometry strategy, 2nd to observe H_2S-releasing enzymes in sperm and describe the effect of exogenous H_2S supplementation on sperm quality, 3rd to track SIRT1 and its targets across the oocyte maturation and finally to investigate the effect of BPS on oocytes and sperm, as well as ovaries and testes, through selected markers. Formally, the study is well-written and contains only few grammatical errors.

The chapter „Material and Methods" contains information about sample (spermatozoa and oocytes) collection and isolation, methods for the evaluation of sample quality as well as clearly defined design of the experiments. Methods used for this study were chosen properly to the aims. However, several methodological recommendations and remarks are summarized below.

PhD student collected a lot of data and results that are presenting in an appropriate form using tables, charts and figures with relevant statistical analysis that are sufficient for this type of work. Presented results demonstrate a new epigenetic marker H3K4me2 which expression is dependent on spermatozoa quality and chromatin immaturity. It was observed that SIRT1 is participating in the spermatozoa maturation and epigenetic and non-epigenetic targets of SIRT1 in oocytes have been also identified. Furthermore, the role of gasotranmitter H_2S, a potent signal molecule modulating protein activity, including SIRT1, has been investigated. It was found out that H_2S slows down sperm capacitation possibly through posttranslational modifications (PTMs) of proteins involved in that process. Finally, endocrine disruptive effect of BPS was confirmed on sperm and oocytes through modification of histone code as well as other PTMs of proteins.

In the "Discussion" author offers logical and substantive analysis of the obtained results in confrontation with other domestic and foreign studies.

In the “Conclusion” chapter PhD student clearly conclude that these epigenetic based markers, sensitive to environmental exposure, might be promising indicator of gametes quality and predictor of fertilization success.

General comments:

1) Page 21: This sentence makes no sense: “The meiosis recombination when the genetic information between homologous chromosomes is exchanged is typical or meiosis.”

2) In “Methods”: Sperm concentration and motility were evaluated subjectively using Makler chamber. However, Makler chamber is only a chamber that is used together with some Computer-assisted sperm analysis (CASA) systems. If you used such CASA system, please mention it within the methods.

3) You used 3 experimental BPS groups in male experiments, but four BPS groups in female experiments. Why?

4) Concerning the measurement of sperm apoptosis, did you assess also the dead sperm or did you distinguished between necrotic sperm and dead sperm that died as a result of apoptosis (the late apoptotic cells that were also Yopro positive)?

5) In sperm chromatin structure assay is mentioned that sperm samples were diluted and immediately frozen in liquid nitrogen. Did you freeze the samples directly in LN_2 or you used the vapours of LN_2 for freezing and then samples were transferred into LN_2 container?

6) The histone detection by flow cytometry was performed according to the study of Li et al. However, the year of this study is missing. Please correct it.

7) In “Methods”, mouse monoclonal and polyclonal antibodies were used for staining of mouse oocytes samples in WB and IF techniques. How did you ensure the true specificity of these antibodies for mouse target epitopes?

8) It is not clear which spermatozoa were used in the method for quantification of H_2S-releasing enzymes. In general, spermatozoa of 3 different species (human, mice and boar) were used in this study. However, it should be clearly mentioned each time within the methods from which species the spermatozoa come.

9) The explanation of the abbreviation "FZ3" is missing within the whole text. Concerning that how was the Zinc signature analysed?

10) The MFI value was used in order to compare the presence of H3K4me2 within the spermatozoa of different quality (Table 1 and 2). However, the proportion (%) of the spermatozoa positive for H3K4me2 within the whole sperm population could be an interesting finding and might be presented in both tables.

11) Reference on the page 75: "Arur S. Signaling-mediated control of cell division: from oogenesis to oocyte-to-embryo development" is incomplete.

Questions

1) Could be H3K4me2 used as a biomarker also for farm animal sperm quality?
2) Is it possible to use H3K4me2 in combination with some non-invasive sperm selection technique and how?
3) Are there any studies focusing on the effect of epigenetics on the reproduction of farm animals?

Conclusion:

I note that Mgr. Miriama Štiavnická presented her abilities and readiness for independent scientific activity in a given research area. The submitted PhD thesis met the stated aims with A (1). From this viewpoint I recommend allowing the submitted thesis to be defended and, in case of its successful defence, I propose to award Mgr. Miriama Štiavnická a scientific academic title „philosophiae doctor" PhD.

In Nitra, August, 19th, 2019 Ing. Jaromír Vašíček, PhD.