Opponent Review of Doctoral Thesis

Thesis title: **Male infertility and DNA germ cell breaks affected by the epigenetic factor PRDM9**

Thesis author: **Fitore Kusari, M.Sc.**

Supervisor: **Ing. Zdeněk Trachtulec, Ph.D.**

Reviewer: **M.Sc. Lukáš Děd, Ph.D.**

Ph.D. study program: **Molecular and Cellular Biology, Genetics and Virology, Charles University in Prague, Faculty of Science**

Thesis background

The presented doctoral thesis mainly address reproductive phenotype profiles of the mice and rat models altered in the function of the hybrid sterility-causing PRDM9 gene. Hybrid sterility constitutes one of the post-zygotic reproduction isolation mechanisms when fertile parental generation gives a rise of the infertile progeny. Doctoral thesis supervisor was previously involved in the studies addressing the identification and establishment of PRDM9 gene as the mice speciation gene (Hst1) among multiple genes present in 255 kb locus at chromosome 17. PRDM9 encodes histone methytransferase acting on H3K4 and H3K36 (me3). During the meiosis it marks so-called recombination hotspots where the DNA recombination takes place at high frequency.

Thesis topic and outputs

The major focus of the presented thesis is the characterization of the reproductive phenotypes of the mice and rat models altered in the function of the hybrid sterility-causing PRDM9 gene. Since the role of the PRDM9 gene during the meiotic process has been largely address by the group and others, author and colleagues logically focus on potential effect of the PRDM9 functional alterations on the post-meiotic events and phenotypes with emphasis on the process of spermatogenesis (including histone-to-protamine transition) and sperm parameters. The major scientific outputs of the thesis are the finding, that PRDM9 protects spermatozoa from the development of nuclear DNA breaks. Thesis further provides genetic evidence based on two laboratory animal species (mice and rat) suggesting that loss-of-function mutations in Prdm9 lead to acephalic spermatozoa syndrome. Author also suggest, that these two findings suggest possible role of the human *Prdm9* in the pathogenesis of the human infertility.

Thesis formal structure

During her doctoral studies, candidate published two original articles related the thesis theme fulfilling the doctoral study program requirements. First (Kusari et al., 2020) was published in the prestigious societal journal *Reproduction* and thesis author is the first author of the published work. Second (Mihola et al., 2021) was published in the high quality general biological scope journal *BMC Biology*, where author is the member of the collective of authors. The scientific outputs of both articles are recapitulated, presented and summarized as substantial body of text constituting presented doctoral thesis, as required by the doctoral study program conditions. Here I have several points which I would like to ask to be addressed during the thesis defense.

Thesis formal structure/ requirements fulfillment

My first point is about the citations of the original articles (Kusari et al., 2020; Mihola et al., 2021) in the thesis text. When Mihola et al., 2021 is cited ten times throughout the text, Kusari et al., 2020 is not cited at all when both are mentioned in section 7. (page 88) as relevant publications. I think it would be helpful if author can cite appropriate publications especially during the presentation of figures and disclose if they were adapted/adopted from relevant publications and which figures (data) are presented only in the thesis without being presented in relevant articles.

My second point here is related to the statistical analysis of the data. In the thesis methodology (section 3.19 Statistical analysis; page 44) is very generally described overall statistical approach, which does not fully correspond at least with the figures adapted/adopted from Mihola et al., 2021 (according to the article methodological section). I suggest candidate to present individual data/figures during thesis defense together with the comprehensive information about concrete statistical/post-hoc tests, which were used to analyze the statistical significance.

My third point here is related to candidate contribution to the individual scientific outputs. According to my opinion candidate fulfilled the requirement to address her contribution to individual scientific outputs of the thesis by the authors' contribution section in relevant articles (Kusari et al., 2020; Mihola et al., 2021). At the same time I suggest to elaborate this issue a bit more during thesis defense (e.g. by the section "my contribution to individual parts of this work" and/or during the presentation of individual data sets) since this requirement is highlighted in the study program conditions for thesis defense.

Thesis review conclusions

To summarize my review: Author fulfilled the requirements of the doctoral study program *Molecular and Cellular Biology, Genetics and Virology at the Charles University in Prague, Faculty of Science* by presenting interesting and scientifically sound results addressing the reproductive phenotypes of the PRDM9 animal models in two original articles (Kusari et al., 2020; Mihola et al., 2021) which were newly addressed and summarized in the presented, comprehensive doctoral thesis *Male infertility and DNA germ cell breaks affected by the epigenetic factor PRDM9.*

Based on my evaluation and conclusion I suggest this thesis to be accepted as a doctoral thesis and after addressing raised points and the successful thesis defense I suggest to confer a scientific degree Ph.D.

Quoestions for discussion

1. PRDM9 would play various roles during the pre-meotic/meiotic/post-meiotic events. Since authors used various animal models I would like to ask author if she can address the potential design of the mice animal model where the alteration of the PRDM9 function would be introduced only in the post-meiotic germ cell population, e.g. describe which transgenic technique strategy might be utilized, its benefits and problematic aspects and what differences from non-post-meiotically targeted models might be expected.

2. The relationship of the quality of the genetic material carried by individual sperm and its reproductive fitness (e.g. motility etc.) is a major topic of current reproductive biomedicine. Since author addressed the coincidence of the DNA breaks and acephalic sperm production in PRDM9 animal models I would like to ask which are the current concepts addressing

the mechanisms linking the quality of the genetic material and sperm reproductive fitness (here with emphasis on sperm LINC complex and/or according to authors´ expertise).

3. Author concludes that findings presented in the thesis raise the possibility that Prdm9 may be a new candidate gene involved in human infertility cases. Since the translational research of the basic reproductive research to reproductive (bio)medicine is issue of great importance I would ask if author and/or whole research group already carried out some bioinformatics preparatory work and/or pilot experiments and if they already have strategy how to proceed in this in future.

In Prague 3.8.2021 ……

M.Sc. Lukáš Děd, Ph.D.