PhD thesis evaluation

This is the evaluation of the thesis for Ph.D. defense of Katarzyna I. Szczerkowska: **Function of Zinc finger protein 644 (Zfp644) in mouse organism.**

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The Ph.D. thesis of Katarzyna I. Szczerkowska is organized around several findings about Zinc finger protein 644 (Zfp644) and molecules functionally associated with G9a/GLP complex, especially with Wiz. Most relevant to the defense of the applicant is her first publication describing mouse model of a disease caused by point mutation in Zfp644 that in human leads to myopia. This is nicely documented example of mice genetical manipulation leading to an elegant murine model of a human disease. Katarzyna I. Szczerkowska is the first author on this paper. This primary finding, presented in this peer reviewed journal, is further examined by two additional mice mutants, in which partial deletion of Zfp644, and mice bearing total deletion are examined to analyze the biological role of Zpf644 in vivo
The thesis is further aimed at addressing the Wiz protein role in embryo as the functional ablation of this gene is lethal. The findings resulted in a second manuscript on which the applicant is a co-author.

From the above it is clear, the thesis topic is a little too complex, which is also complicated by the fact that the *Zfp644* $^{\Delta 8}$ *mutant has a stronger phenotype than the mutant with the gene ablation*. It would be helpful, should the thesis be written in more comprehensive way, or at least without numerous typos, logical gaps, inconsistences. I will not comment on this further, but even a spell check would help, sometimes.

Specific questions:

There are differences between phenotypes of wild type animals, and mice expressing modified versions of ZNF644 gene: *Zfp644*^{S673G}, *Zfp644* $^{\Delta 8}$, *Zfp644*$^{-/-}$. It would be helpful for the discussion to include a table summarizing the observed differences in the phenotypes of these strains. How could be these differences explained on molecular level, and can some procedures to investigate this be proposed? Has some progress been done in this direction?

The fertility of *Zfp644* $^{\Delta 8}$ females was rescued by progesterone substitution, but not estrogen/progesterone substitution, that seems to have contraceptive effect (also in wt animals) (Figure 4.19. and a corresponding text). Is there any hypothetical explanation? Moreover, the situation differs from *Zfp644* $^{-/-}$ mice, that do not require hormonal substitution, as they are fertile. This is an interesting observation that should be discussed as well (see above comment).

The lack of good antibodies is a common problem. However, the author claims that production of monoclonal antibodies was not successful. Was the serum of the mice tested itself, or was production of polyclonal sera considered in another animal?

Minor point:
The author claims she was involved in "writing of the original manuscript and creating the figures in the articles". However, this is not in accordance with the "authors contribution" in the text. These things should be in accord. However, this does not undermine the overall value of the thesis.

I recommend that Katarzyna I. Szczerkowska may obtain a Ph.D. title based on the publications, and this thesis.
Doporučuji, aby Katarzyna I. Szczerkowska na základě publikací a této diplomové práce získala titul Ph.D.

Doc. MUDr. Jaroslav Blahoš, Ph.D.