**Abstract**

ZNF644 (Zinc Finger Protein 644) is a C2H2 zinc finger gene encoding a putative transcription regulator, of which a point mutation (S672G) is associated with inherited high myopia in humans. It is also described to be a partner of the G9a/GLP (G9a- euchromatic histone-lysine *N*-methyltransferase 2, EHMT2; GLP - euchromatic histone-lysine *N*-methyltransferase 1, EHMT1) complex, known for its essential role in histone methylation, specifically H3K9me1and H3K9me2. It was reported that another transcription factor, WIZ (Widely-Interspaced Zinc Finger-Containing Protein), can bind to this complex and cooperate in gene silencing simultaneously.

In order to study *Zfp644* impact on myopia, we generated a mouse model, $Zfp644^{S673G}$ that mimics human mutation. In addition, a mouse with a persuasive truncated form of the protein, $Zfp644^{\Delta 8}$ was created. Both mouse models went through an examination of retinal function and morphology. Moreover, with use of ultrasonography, different ocular parameters were examined. We conclude, that *Zfp644* gene is causative for myopia in mice. Further examinations of $Zfp644^{\Delta 8}$ animals show severe symptoms in metabolism and female fertility. To describe the impact of Zfp644 in mouse fertility we performed various experiments including analysis of expression of *Zfp644* in reproductive organs, breeding performance, ovarian morphometry and estrus cycle. Additionally, a full knockout mouse model named $Zfp644^{-/-}$ was prepared and analyzed together with $Zfp644^{\Delta 8}$ in the fertility study. The levels of hormones crucial for correct estrus cycling, in each of the phases, was analyzed. We also followed the development of mammary gland growth during puberty and during pregnancy. We found striking differences in $Zfp644^{\Delta 8}$ females, when compared to $Zfp644^{-/-}$ and control animals. The studies were complemented by the mammary gland organoids assays. Furthermore, a transplantation of ovaries from $Zfp644^{\Delta 8}$ homozygous to control animal; and vice-versa was performed. We rescued homozygous $Zfp644^{\Delta 8}$ ovaries transplanted to control animals. Ovaries were fully functional and females were able to successfully breed and deliver multiple litters. However, $Zfp644^{\Delta 8}$ homozygous females after transplantation of ovaries remain sub-fertile. To fully rescue the subfertility phenotype in $Zfp644^{\Delta 8}$ females, we decided to apply hormone replacement therapy. We were able to successfully breed $Zfp644^{\Delta 8}$ homozygous females and generate the viable offspring when applying progesterone therapy. Taken together,

our most recent data shows strong aberration in female sex hormone homeostasis, which seems to be the cause of the female infertility we observed.

To complement our study by further exploration of G9a/GLP complex function, we expanded our research by another transcription factor, working simultaneously with G9a/GLP complex and ZNF644, WIZ protein. Similar to *Zfp644* studies, we investigated a role of *Wiz* knockout in mouse. Unlike *Zfp644*, functional ablation of *Wiz* causes late embryonic lethality. However not as early as *G9a* nor *GLP* knockout mice. That might suggest higher importance of Wiz protein when compared to Zfp644 in the methylation complex however not crucial for methylation in early embryonic stages. Morphological changes were deeply analyzed in embryos between E14.5 to E18.5. The most severe malformations include a shorter snout, cleft palate and cleft eyelids. Moreover, we analyzed the histone methylation to understand the impact of *Wiz* knockout on G9a/GLP function. Based on the data, we conclude that the histone methylation pattern is suppressed. Our data strongly suggest, that Wiz plays an important role in the G9a/GLP methylation complex, especially in craniofacial development.

Taken together, this work presents a novel data that could be valuable for further studies on ZNF644, WIZ and G9a/GLP complex. The close similarity between human and mouse Zinc Finger Protein 644 implies the possibility for translation of our study on myopia to human medicine in the future. Further research on the G9a/GLP methylation complex and its action in the organism, influenced by ZNF644 and WIZ is necessary, however, we believe that presented data might shed a light on molecular mechanism of this complex.

**Key words**: Zfp644, ZNF644, WIZ, G9a, GLP, histone methylation, transcription factor, vision, fertility, development