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THE MUTATION OF FSHR AFFECTS REPRODUCTIVE PHYSIOLOGY AND GROWTH IN ZEBRAFISH *Danio rerio*

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Introduction

Fsh is a pituitary gonadotropin that regulates both gonadal functions, gametogenesis and steroidogenesis, in fish. The biological actions of Fsh are mediated through its binding to a membrane receptor (Fshr) that belongs to the superfamily of the G protein-coupled receptor (GPCR). Previous reports on *in vivo* disruption of the Fshr gene showed that Fsh signaling pathway was dispensable for male fertility but was required for oogenesis. In the present study we generated new mutant zebrafish lines to further investigate the role of *fshr* in reproductive and growth performances.

Methods

We disrupted the *fshr* gene in zebrafish using the CRISPR/Cas9 method. We selected three CRISPR sites in the 10th exon of the gene to cause a large deletion easily detectable by a PCR-based genotyping method. Two mutant lines were generated from independent founders mated with transgenic *vasa:eGFP* females. The mutations were characterized by DNA sequencing in both lines. The deletions led to predicted truncated proteins unable to anchor to the membrane and to transduce Fsh signal.

Results and Discussion

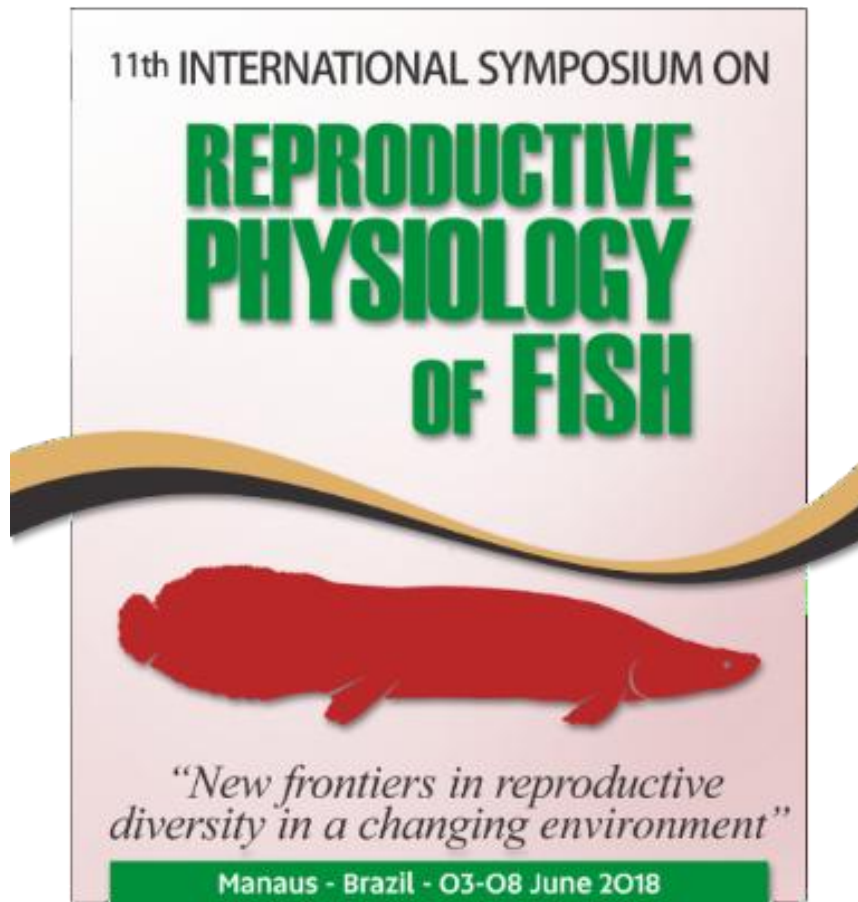
Heterozygous mutant males and females developed an apparently normal gametogenesis and were fertile. However heterozygous zebrafish population (*fshr*^{+/-}) showed a female biased sex-ratio compared to the non mutated siblings. At 27 days post fertilization heterozygous mutants showed higher relative abundance of the gonadal aromatase gene (*cyp19a1a*) and increased levels of transcripts involved in granulosa cell differentiation (*foxl2a2*, *gsdf*, *fshr*, *sox9a*). In addition, transcripts accumulated during late oocyte growth (*vasa*, *sox9b* and *nanos2*) were also detected at higher levels. The relative abundance of *sycp1* was unchanged indicating that meiosis initiation was normal. In contrast, in homozygous mutant meiosis was altered as revealed by decreased expression levels of *sycp1*. In addition, oocytes did not progress beyond the pre-follicle phase of primary growth. All adult homozygous mutants became males suggesting that impaired oogenesis during early ontogenesis caused sex reversal.

Additional new phenotypes were observed in the homozygous males including lower sperm count and viability. This could result from 11-KT deficiency as revealed by decreased expression levels of *cyp11b2*. Perturbation of steroidogenesis could also explain the increased body weight and length observed in homozygous mutants.

Conclusion

The marked phenotypic perturbations induced by the Fshr mutation confirm that Fsh and Lh have distinct functions. We showed that the loss of one *fshr* gene copy favours the differentiation of granulosa cells and oocyte growth. Fsh signalling pathway is dispensable for Sertoli cell differentiation but remains important for sperm quality and excretion.

PROGRAM AND ABSTRACTS



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