

POSTER 1

RETINOIC ACID INDUCES MEIOSIS AND REGULATES EXPRESSION OF GENES RELATED TO SEX DETERMINATION IN MEDAKA

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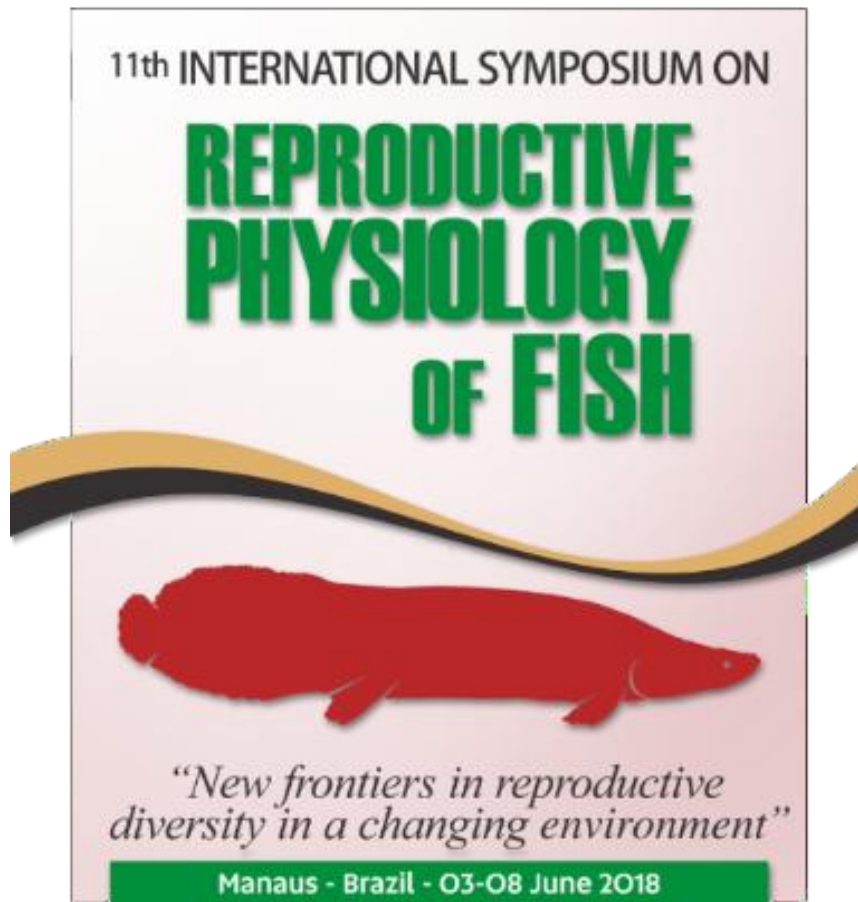
Introduction : Sex determination (SD) is a complex and diverse developmental process that leads to the decision whether the bipotential gonad anlage will become a testis or an ovary. The timing of meiosis entry is one of the first recognizable differences between male and female in vertebrates. The germ cells go into meiosis first in females and later in males. In mammals, this event has been shown to be regulated by retinoic acid (RA). This polar small molecule induces in the germ cells the expression of the pre-meiotic marker *Stra8* (stimulated by retinoic acid gene 8), which is necessary for meiosis initiation. Interestingly, genome analyzes have shown that the majority of fish (including medaka) lack *stra8*, adding a question mark to the role of RA in meiosis induction in this group. We investigated in medaka (*Oryzias latipes*) a possible signaling function of RA during the SD period in embryos and in reproductively active gonads of adults.

Methods : We generated transgenic medaka reporter lines of the main genes involved in RA metabolism (*aldh1a2*, *cyp26a1* and *cyp26b1*), and a transgenic RA-responsive reporter line containing 12 copies of the RARE together with eGFP (12XRARE). In addition, the *cyp26a1* gene was disrupted using the TALEN method. We performed several RA treatments in embryos, cells and testis organ cultures, and checked for gene expression by qRT-PCR.

Results and Discussion : RA mediated transcriptional activation in germ cells of both sexes much earlier than the SD stage, however, no such activity was noted during the critical stages of SD. In adults, expression of the RA metabolizing enzymes indicates sexually dimorphic RA levels. In testis, RA acts directly in Sertoli, Leydig and pre-meiotic germ cells. In ovaries, RA transcription regulating activity is highest in meiotic oocytes. Expression analyzes of embryos treated with exogenous RA showed induction of *dmrt1a* at the gonad levels and an increase of *amh* levels. Both genes are known to be involved in the regulation of germ cell proliferation and differentiation. Disruption of the *cyp26a1* gene leads to an early entry of meiosis and oocyte formation in XY medaka larvae, as well as upregulation of *dmrt1a* in adult testis and downregulation of *foxl2* in adult ovary.

Conclusion : RA is important in meiosis induction and gametogenesis in adult medaka. Moreover, contrary to common expectation, RA induces sex related genes that are involved indirectly in meiosis inhibition. We showed for the first time that RA can be involved in induction of meiosis entry, and regulates genes related sex determination and differentiation depending on the sex and the developmental stage in a *stra8*-independent model organism.

PROGRAM AND ABSTRACTS



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