MOLECULAR CHARACTERIZATION OF TESTIS DIFFERENTIATION IN THE SIBERIAN STURGEON, *ACIPENSER BAERII*

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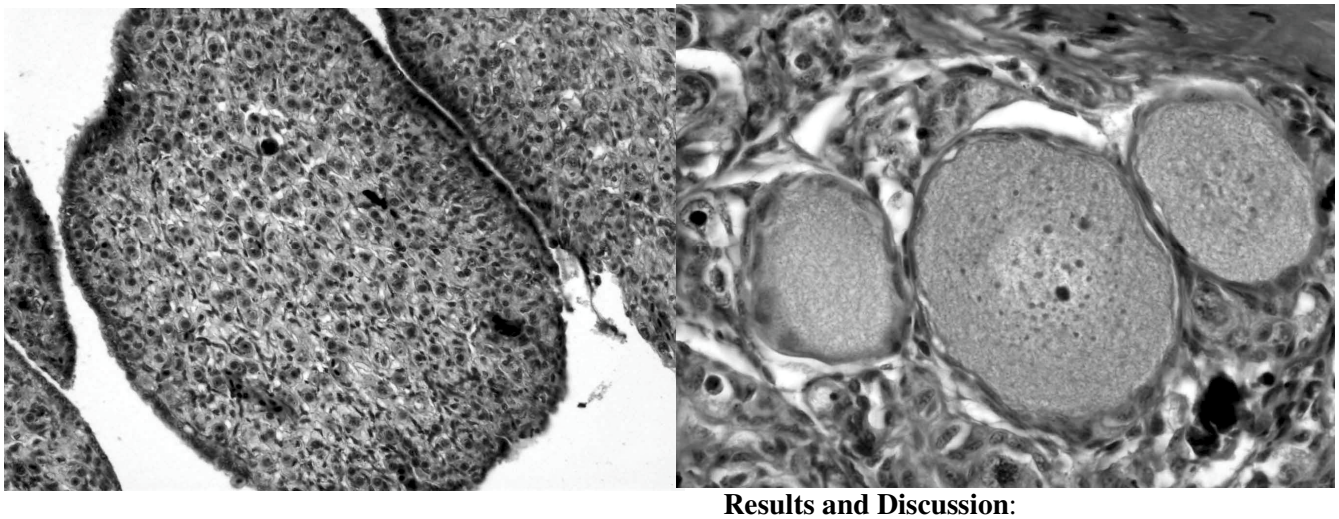
Introduction:

The morphological differentiation of vertebrate gonads is preceded by molecular changes that are not completely understood in the different vertebrate taxa. The most studied genes involved in the masculine pathway were Sox9, Dmrt1, Amh, Nr5a1, NrOb1 and Wt1 [1]. Besides transcription factors and other proteins, the endogenous steroids have been accepted as key players for gonad sex differentiation in fishes. In fact, *cyp19a1* (gonadal aromatase) is a key gene for ovarian differentiation [2], and androgens seem to have a key role during testis determination-differentiation in temperature-sensitive species [3]. However, the molecular mechanisms underlying gonad differentiation in phylogenetically ancient fishes as sturgeons remain poorly understood. As the time of sex differentiation was not reported for the species in rearing conditions in Uruguay, we started by an identification of this period by gonad morphology. For the molecular characterization of testis differentiation seven genes were studied, including testis differentiation markers as *dmrt1, ar*, *sox9*, *igf1* [1][4], and some markers of steroid synthesis (i.e. *cyp17*, *star*) and *lh.* A complete study on sex differentiation was difficult due to the scarce information on genes markers of gonad development in sturgeons, specially the ovarian markers. In this work *dmrt1*, *cyp17* and *star* sequences were characterized for *A. baerii*. The expression of genes was studied around the sex differentiation period as well as in already differentiated fish.

Methods:

Siberian sturgeons were obtained from producers of Uruguay. One gonad was fixed for histology, and the contralateral gonad was frozen in liquid nitrogen and transferred at -80ºC until RNA extraction. For gene characterization, primers were designed from fish data bases at conserved sequence regions and used to amplify cDNA of post-differentiated testis. The fragments amplified were sequenced, and blasted in order to confirm their identity. The partial cDNAs sequences encoding for *dmrt1* (HQ110106), *ar* (HQ110107), *cyp17* (HQ026486) and *star* were characterized. Primers for qPCR were designed based on the gene sequences characterized here (*dmrt1*, *cyp17*) and on sequences previously submitted to GenBank for *ar* (DQ388357.1), *sox9* (EU241882.1), *igf1* (FJ428828.1), *lh* (AJ251656.1). For qPCR we studied 12 undifferentiated gonads, 10 just differentiated ovaries and 4 just differentiated testis (16 months old), and 4 ovaries at stage III (oocyte diameter of 2 mm) using 18S (AY904445.1) or ß-actin (FJ205611.1) for standardization. The validity of qPCR was confirmed by analysis of melting curves and checking amplified fragments in agarose gel.

Fig.1. Histological sections of gonads of undifferentiated fish (left) and just differentiated females (right).



Results and Discussion:

The histological analysis of gonads collected from 22 animals at age 16 months showed that 55% sampled fishes had undifferentiated gonads (Fig. 1 A) and 45% had differentiated ovaries with early pre-vitellogenic oocytes (Fig. 1 B). Four fish samples at the age 16 months had differentiated testes. This differs from data reported for other sturgeon species in which the differentiation occurs at 9 months [5].

Comparative expression in ovaries and testis of 16 months old showed a male up-regulation of 67 fold for *igf1* ($p<0.0001$) and of more than 200 fold for *dmrt1* ($p<0.001$), *ar* ($p<0.001$), *lh* ($p<0.001$), *cyp17* ($p<0.001$). *star* was not dimorphic at this stage. This trend was also observed when just differentiated testis was compared to ovaries at stage III, except for *sox9* and *star* that were slightly over-expressed in females.

The undifferentiated gonads showed low level of expression in all genes analyzed. For undifferentiated gonads the trends of *dmrt1*, *ar*, *lh* and *cyp17* were similar, with some fish showing high levels of expression and the others very low expression level, suggesting over expression in those probably undergoing male differentiation. Among the genes selected the transcription factor *dmrt1* seems to play a central and conserved role in fish testis differentiation [6]. The higher expression of this gene in Siberian sturgeon testis suggests that the function of *dmrt1* appeared already in this ancient fish and was conserved in teleost. Androgens play essential roles in sex differentiation and sexual maturation in vertebrates and their actions are mediated through androgen receptors that seem important during *A. baerii* testis differentiation. The *sox9*, a direct downstream target of SRY in mammals [7], seems not to play and essential role in testis development of chondrostean fish. The steroid synthesis capacity seems to be higher in just differentiated testis when compared to ovaries, but more efforts must be made to conclude if undifferentiated gonads are able to produce steroids.

Conclusion:

Four genes (*dmrt1*, *ar*, *lh, cyp17)* can be considered as potential markers of testis differentiation before we can identify sex of Siberian sturgeon juveniles by the testis morphology.

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