

Dmrt1 and Cyp17a1 Protein Detection and Relative Quantification in Best Beluga Hybrid Sturgeon Gonads

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Abstract

Sturgeons are highly valuable species from both a conservation and economic point of view. Their very sought-after caviar extracted from mature females has both a rich culture around it and opens the way for interesting research. Because of the advanced age at which females and males could be identified in aquaculture it is important to develop a set of molecular markers that could make the distinction easy. For this, a better understanding of the sexual development of sturgeons is necessary. We selected the Dmrt1 (doublesex and mab-3 related transcription factor 1) and the Cyp17a1 (17 α -hydroxylase) markers with the scope of investigating the gonad development of sturgeon individuals. Both markers appear to be expressed in the gonad but no statistically significant difference in expression is observed between females and males. This could mean that the markers are equally involved in female and male sexual development at the stage of sampling. This study paves the way for a better understanding of sturgeon sexual development.

Keywords: gonad development, Western Blot, sturgeons, aquaculture.

1. Introduction

Sturgeons are threatened in their natural habitat and just one (*Acipenser ruthenus*) of the four sturgeons species found in the Danube has the status of vulnerable while the rest are critically endangered (*Acipenser gueldenstaedtii*, *Acipenser stellatus* and *Huso huso*) [1]. Sturgeon aquaculture has become important because of the high economic value of sturgeon products such as meat and caviar. For a better yield and faster maturation times, one of the practices in aquaculture is to obtain hybrid sturgeon individuals that mature faster and have good quality caviar, one example of this is the Best Beluga (♀ Bester (♀ *H. huso* \times ♂ *A. ruthenus*) \times ♂ *H. huso*) sturgeon hybrid.

The gonad development study of individuals assists aquaculture with the scope of understanding the sexual maturation of sturgeons and identifying a method of accurately sex individuals at an early stage of development. For this we selected two markers, Dmrt1 (doublesex and mab-3 related transcription factor 1) and Cyp17a1 (17 α -hydroxylase) that have been studied in relation to sturgeon sexual development.

Dmrt1 is a transcription factor involved in sexual differentiation observed in different species, from mammals [2], birds [3], reptiles [4], amphibians [5] and fish [6]. This protein has been shown to be essential in testicular differentiation in mammals [2], birds [3] and some fish species [6]. In sturgeons (*Acipenser sinensis*), the Dmrt1 protein is important in the early differentiation of the testis [7].

Cyp17a1 is an enzyme involved in the synthesis of steroid hormones which in turn are key regulators

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of growth, development and reproduction in vertebrates [8]. It has been detected in mammals [9] and in fish species [8, 10]. In sturgeons the mRNA transcript of *cyp17a1* was found in both males and females with a significantly higher expression in males [11].

The aim of this study is to investigate the relative expression of Dmrt1 and Cyp17a1 in Best Beluga hybrid sturgeon gonads.

2. Materials and methods

Ten Best Beluga hybrid sturgeons from aquaculture (21 months old, five males and five females) were anesthetized in a water bath with 0.3 mL/L 2-phenoxyethanol and then sacrificed. The gonads were sampled, transported on ice to the laboratory and stored at -80°C .

The gonad homogenates were prepared as 1 g of tissue to 10 volumes of buffer (0.1 M TRIS-HCl, 5 mM EDTA, pH 7.4) by sonication on ice using the UP100H (Hielscher) sonicator. After a 10000 rpm centrifugation for 30 minutes at 4°C the supernatant was stored at -80°C until further analysis.

The protein concentration was determined using the Lowry method [12] with BSA (Bovine Serum Albumin) as standard.

Protein samples of 50 μg were migrated by SDS PAGE in a 12% polyacrylamide gel and transferred onto a 0.2 μm pore PVDF membrane. For detection of Dmrt1 the membranes were incubated with antiDmrt1 rabbit antibody of a 1:1000 dilution, for Cyp17a1 a 1:500 dilution of antiCyp17a1 rabbit antibody was used while a 1:10000 dilution of anti β -actin mouse antibody was used for the β -actin protein. The Western Breeze Chromogenic Immunodetection kit (Thermo Fisher Scientific) with secondary antibody (coupled with alkaline phosphatase) and with BCIP/NBT chromogenic substrate was used to develop the membrane. The immunoreactive bands were visualized and quantified using the Image Lab 5.2.1 software (Bio-Rad).

For data normalization the β -actin protein was used. The values were expressed as arithmetic means with standard deviations and the differences between males and females were analyzed using Student's t test with a p value of less than 0.05 considered significant.

3. Results and discussion

For Dmrt1 the Western Blot shows that the antibody is not specific (Figure 1a) while the band of 27 kDa attributed to the Dmrt1 protein is present. No statistically significant difference in the relative expression of Dmrt1 between males and females was observed for 21 months old individuals (Figure 1b).

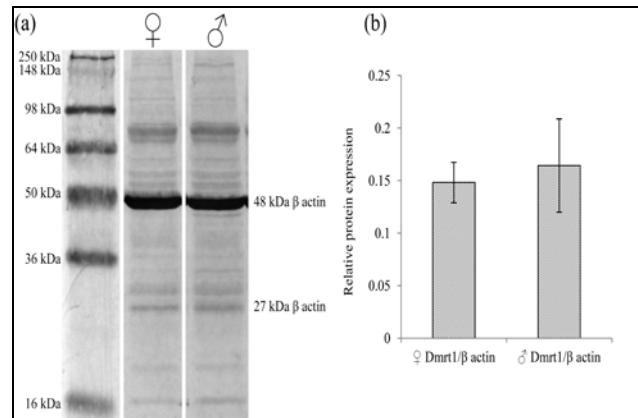


Figure 1. Detection of Dmrt1 in Best Beluga hybrid sturgeon gonads. (a) Western Blot analysis of Dmrt1 protein in gonads of males (♂) and females (♀). (b) Difference ($p \leq 0.05$) in relative protein expression of Dmrt1 between males and females (mean \pm SD).

It has been reported that *dmrt1* presents no statistically significant difference in expression between male and female gonads in case of *Scaphirhynchus platyrhynchus* mature individuals [13], and *H. huso* individuals with small oogonia in females and immature testicles in males [11]. These results are backed up by our own observation where for the 21 months old individuals no statistically significant difference is observed between males and females in case of the Dmrt1 protein. It has also been observed that in case of *A. ruthenus* individuals with spermatocytes and previtellogenic oocytes the expression of *dmrt1* was significantly higher in males than in females [14]. In case of *A. sinensis* the expression of *dmrt1* has been reported to be higher in males than in females especially at three and four years and that Dmrt1 is also present in higher quantity for testis [7]. These different results could be the cause of the different species investigated.

The fact that Dmrt1 is detected in both testis and ovaries with no difference in expression between them in the Best Beluga hybrid individuals is

probably due to its involvement in both testicular and ovarian development at 21 months. Another explanation could be that this is the basal level of expression before (or after) the stage of high *Dmrt1* expression in males.

In case of *Cyp17a1* the Western Blot results reveal that the antibody is not specific but the band of 56 kDa attributed to the *Cyp17a1* protein is present (Figure 2a). It is observed that there is no statistically significant difference between the relative expression of *Cyp17a1* between females and males (Figure 2b).

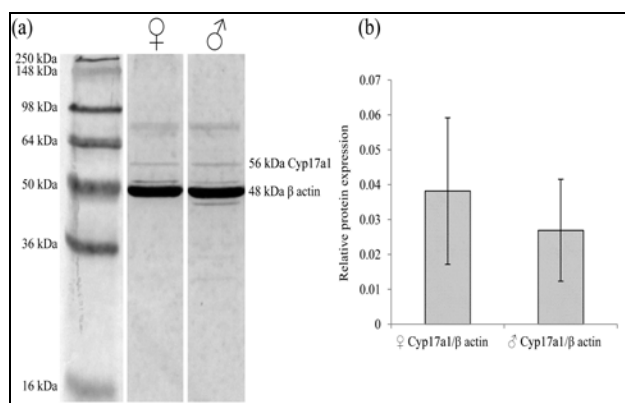


Figure 2. Detection of *Cyp17a1* in Best Beluga hybrid sturgeon gonads. (a) Western Blot analysis of *Cyp17a1* protein in gonads of males (♂) and females (♀). (b) Difference ($p \leq 0.05$) in relative protein expression of *Cyp17a1* between males and females (mean \pm SD).

For *H. huso* individuals with oogonia and immature testicles it has been reported that there is a higher expression of *cyp17a1* in testicles than in ovaries [11]. The *Cyp17a1* protein is an enzyme that converts progesterin into androgens and it has been reported that it is controversial whether androgens are involved in gonad differentiation or in gonad development [15] at the onset of gametogenesis. Because the expression of *Cyp17a1* is present at 21 months for the Best Beluga individuals it could be argued that it is involved in the gonad development or in the onset of gametogenesis for both sexes.

4. Conclusions

Both *Dmrt1* and *Cyp17a1* were detected in gonad homogenates which is an indication of their involvement in sturgeon gonad development. This study paves the way for a better understanding of sturgeon sexual development.

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