Effects of estradiol-17β and 17α-methyltestosterone on gonadal sex differentiation in the F₂ hybrid sturgeon, the bester

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ABSTRACT: The effects of giving oral estradiol-17β (E₂) and 17α-methyltestosterone (MT) on gonadal sex differentiation in the F₂ hybrid sturgeon, the bester (Huso huso female × Acipenser ruthenus male), are investigated. Giving E₂ at 10 μg/g diet to fish from 14 months until 31 months of age induced incomplete feminization and resulted in approximately 40% abnormal ovary development in which oocytes were observed without ovarian lamellar structures and gonadal shape was similar to normal testis. Giving MT at 25 μg/g diet for the same duration failed to induce masculinization, and resulted in approximately 30% undeveloped gonads even at 30–37 months of age. In contrast, E₂ and MT at only 1 μg/g diet given from 3 to 18 months of age was sufficient to induce feminization and masculinization, respectively. In these fish, feminization and masculinization were observed at 9 months, when most putative ovaries and testes were histologically distinguishable by the shape of the gonadal surface. These results indicate that sex reversal can be induced in these fish by hormone treatment that is started at 3 months age, before morphological differentiation occurs on the stroma of the gonads.

KEY WORDS: estradiol-17β, hormone treatment, 17α-methyltestosterone, sex differentiation, sex reversal, sturgeon.

INTRODUCTION

Controlling sex differentiation by giving sex steroids is useful under certain culture strategies for several fish species. In combination with chromosome manipulation, hormone treatment has been used efficiently to produce an all-female population of salmonids.1,2 Fish breeders and culturists may want to breed males and females separately or to achieve a monosex culture according to biological or economic traits.1,3

In the sturgeon, females are more valuable than males because they produce roe for black caviar. The decline of caviar fisheries in the Caspian Sea,4 coupled with strong international demand for caviar, has created a potential market for farmed caviar. Thus, sex control, in general, and production of all-female populations, in particular, are of interest to commercial sturgeon aquaculturists.5

Most important to the successful induction of sex reversal by hormones are: (i) the animal’s age at the start of treatment; (ii) the duration of treatment; and (iii) the dose and type of hormones used.6 To date, much of the research in this area has involved Teleostei; data are very limited for sex reversal in non-teleost fish. For the Chondrostei, treatment with estrogen and androgen has been reported only in the sterlet sturgeon Acipenser ruthenus,7 and the paddlefish Polyodon spathula.8 We previously described the process of gonadal sex differentiation in the F₂ hybrid sturgeon, the bester.9 In the F₂ bester, active mitotic and meiotic phases of germ cells were not observable in ovaries even by 16 months of age, and some of the testes showed initiation of spermatogenesis after 21 months of age. However, ‘uneven’ and ‘smooth’ types of gonads were distinguishable as early as 6 months of age.10 In the present study, we examined hormone treatments that started either before the...
Hormone treatment

In experiment 1, E2 and MT were given to fish from 14 until 31 months of age at 10 μg/g diet and 25 μg/g diet, respectively. In experiment 2, both hormones were given from 3 to 18 months of age at 1 μg/g diet. In both experiments, the steroids were dissolved in ethanol and mixed into dry trout food. The ethanol was then evaporated at room temperature for more than 48 h. Control diets comprised the dry food mixed only with ethanol.

Histological observations

In experiment 1, gonads of 86 (control; 27, E2 treatment; 21, MT treatment; 38) fish aged 30 to 37 months were biopsied. In experiment 2, 90 (30/group) fish were killed from 9 and 11 months of age to classify the gonads into ‘uneven’ or ‘smooth’ type according to our previous study, and 90 (30/group) fish were sampled from 18 to 22 months to examine the sex ratio of each group. Body length and body weight were recorded before the samples were excised and fixed in Bouin’s fluid. Fixed tissues were processed for routine paraffin embedding, and 6-μm serial transverse sections of the gonads were stained with Delafield’s hematoxylin and eosin.

Statistical analysis

Fork length data were analyzed in each group by one-way ANOVA followed by Fisher’s Protected Least Significant Difference test. P values of less than 0.05 were taken as significant.

RESULTS

Experiment 1

In control fish, the ovary and testis were distinguishable and the sex ratio was approximately 1:1 (Fig. 1). In ovaries, ovarian lamellae were well developed and oocytes at the peri-nucleolus stage were observed in most fish (Fig. 2a). In testes, the gonadal surfaces were smooth and bulgy. The testicular portion was filled with cysts containing spermatogonia, and spermatogenesis was observed in some fish (Fig. 2b).

In E2-treated fish, testes were not observed, and numbers of normal and abnormal ovaries were roughly equal at 30–37 months of age (Fig. 1). In normal ovaries, ovarian lamellar structures and oocytes at the peri-nucleolus stage were similar to different...
those in control ovaries (Fig. 2c). The number of oocytes at the peri-nucleolus stage varied between fish within each group. In abnormal ovaries, some oocytes at the peri-nucleolus stage lacked ovarian lamellar structures, and gonadal surfaces were smooth, similar to control testes (Fig. 2d).

In MT-treated fish, gonads were classified into five types (Fig. 1). Ovaries were classified as normal or abnormal, similar to those in E2-treated fish, whereas testes were classified as normal or undeveloped. The structure of 'normal' testes was the same as that of control testes; however, 'undeveloped' testes were much smaller and contained only a few germ cells (Fig. 2e). In the remaining fish, gonads were quite small and germ cells were not observed; therefore, the gonadal type was classified as indistinguishable (Table 1).

**Experiment 2**

In fish aged 9–11 months, gonads were roughly classified into two types. ‘Uneven’ gonads were characterized by invagination in the stroma and the presence of a few clusters of germ cells and somatic cells immediately below the epithelium (Fig. 3a,c). ‘Smooth’ gonads were characterized by lack of invagination and by a few clusters of germ cells and somatic cells located deep in the stroma (Fig. 3b,d). In both types of gonad, the number of somatic cells supporting the germ cells varied between fish within each group. However, MT-treated fish also exhibited a few ‘intermediate’ gonads in which the germinal epithelium was invaginated into stroma, as in the uneven type, and a few clusters of germ cells were localized deep in the stroma, similar to the smooth type (Fig. 3e). By contrast, the gonads of some fish were quite small and germ cells were not observed; therefore, the gonadal type was classified as indistinguishable (Fig. 3f). The proportion of uneven, smooth, intermediate and indistinguishable gonads was 13:13:0:4 in control, 28:1:0:1 in E2-treated and 0:22:3:5 in MT-treated fish (Fig. 4).

At 18–26 months, the ovary and testis were distinguishable and the sex ratio was 18:12 in control fish. By contrast, 97% of the gonads were differentiated into ovary in the E2-treated fish, and 93% into testis in the MT-treated fish (Fig. 5).

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**Table 1** Growth and survival rates of each fish group in experiment 1

<table>
<thead>
<tr>
<th>Steroid treatment</th>
<th>No. fish examined</th>
<th>Growth in FL ± SD (mm)</th>
<th>Survival rate %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial size</td>
<td>Final size</td>
</tr>
<tr>
<td>Control</td>
<td>27</td>
<td>284.6 ± 37.2</td>
<td>477.5 ± 67.6</td>
</tr>
<tr>
<td>E2</td>
<td>21</td>
<td>284.6 ± 37.2</td>
<td>431.2 ± 66.7*</td>
</tr>
<tr>
<td>MT</td>
<td>38</td>
<td>284.6 ± 37.2</td>
<td>424.4 ± 67.0*</td>
</tr>
</tbody>
</table>

Asterisks indicate statistically significant differences from control group. E2, estradiol-17β; MT, 17α-methyltestosterone.
Fig. 2  Gonads of the F₂ boster in experiment 1. (a) Ovary of a control fish at 32 months. (b) Testis of a control fish at 30 months. Early spermatogenesis can be seen (inset). (c) Ovary of an estradiol-17β (E₂)-treated fish at 35 months (normal type). (d) Ovary of an E₂-treated fish at 32 months (abnormal type). (e) Testis of an 17α-methyltestosterone (MT)-treated fish at 37 months (undeveloped type). (f) Indistinguishable gonad of an MT-treated fish at 37 months. o, oocyte at peri-nucleolus stage; sg, spermatogonia; sc, spermatocyte; st, spermatid; g, germ cell. Scale bar = 50 μm.
Fig. 3  Gonads of the F₂ bester at 9–11 months in experiment 2. (a) Uneven type gonad of a control fish at 9 months. (b) Smooth type gonad of a control fish at 9 months. (c) Uneven type gonad of an estradiol-17β (E₂)-treated fish at 9 months. (d) Smooth type gonad of an 17α-methyltestosterone (MT)-treated fish at 9 months. (e) Intermediate type gonad of an MT-treated fish at 9 months. (f) Indistinguishable gonad of an MT-treated fish at 11 months. g, germ cell; v, blood vessel; i, invagination of the germinal epithelium; e, germinal epithelium. Scale bar = 50 μm.
In experiment 1, both E2 and MT induced abnormal ovaries in which some oocytes at the perinucleolus stage lacked ovarian lamellar structures and gonadal surfaces were smooth like the testes. No apparent morphological differences in either ovaries or testes were detected between control animals and the hormone-treated groups (Fig. 6). Nor were any significant differences observed in the growth (Fig. 7) or survival rates between the controls and the treated fish.

**DISCUSSION**

In experiment 1, both E2 and MT induced abnormal ovaries in which some oocytes at the perinucleolus stage lacked ovarian lamellar structures and gonadal surfaces were smooth like the testes.
of control fish. Similar phenomena have been noted in coho salmon. In one fish, an abnormal ovary developed into an intersex gonad that contained testicular and ovarian tissue (data not shown). The appearance of hermaphrodites or intersex fish is often observed in some teleosts after treatment with sex steroids. This may be the result of incomplete treatment. In experiment 1, although E2 induced incomplete feminization, MT failed to induce masculinization. It seems likely that these differences are related to the onset of ovarian and testicular differentiation. In the F2 baster, meiosis of germ cells takes place earlier in ovaries than in testes, as for many teleosts, therefore, in order to reverse the gonadal sex effectively, treatment for masculinization must start earlier than for feminization. The roughly 30% of undeveloped gonads induced by MT and the low survival rate in E2-treated fish were probably caused by overdosage. A high dose of MT induces sterile gonads in several teleosts, and E2 at 50–100 μg/g diet results in inhibition of gonadal development in juvenile sterlet sturgeon. As the number of oocytes at the peri-nucleolus stage varied widely between fish within each group, it is not clear whether steroids affected the proliferation and/or development of oocytes.

In experiment 2, feminization and masculinization were induced by E2 and MT, respectively. Nakamura et al. concluded that the so-called critical period for hormone-induced sex reversal is synchronous with physiological sex differentiation of the gonad, and that the period of physiological sex determination in germ cells occurs before morphological differentiation. In experiment 2, E2 induced ‘uneven’ gonads and MT induced ‘smooth’ gonads at 9–11 months. These results are in agreement with previous histological observations of gonadal sex differentiation in the F2 baster, which suggested that uneven and smooth gonads are putative ovary and testis, respectively. Because of the difference in hormone concentrations, we could not compare exactly the results of experiments 1 and 2. But it seems likely that starting hormone treatment in fish aged 14 months, as in experiment 1, is too late to induce sex reversal effectively because morphological differentiation of the stroma of the gonads is distinguishable as early as 6 months of age.

In experiment 2, one fish in the E2-treated group had testis and one fish in the MT-treated group had ovaries. These differences in hormone response in the F2 baster may be due to hybridization itself. In the F2 baster, ovary and testis develop at a similar rate in most fish, but develop faster or slower in a few specimens, and a similar variation in the growth rate of fish has also been reported. It seems likely, therefore, that some variation in response to hormone treatment can be expected as a result of the natural variation in development rates. For both uneven and smooth gonads at 9–11 months, the number of somatic cells supporting the germ cells varied widely between fish in each group, therefore it is not clear whether the steroids affected the proliferation and/or development of these somatic cells. In experiment 2, no apparent morphological differences in either ovaries or testes were detected between the control fish and the hormone-treated fish. Whether these treated fish can mature functionally should be the focus of future studies.

In conclusion, our observations of hormonal sex control in the F2 baster indicate that it is possible to induce sex reversal by hormone treatment if treatment is started before morphological differentiation occurs on the stroma of gonads in the putative ovary of this species.

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REFERENCES


