Short Paper

A Study of Susceptibility of Sex Reversal after a Single 2-Hour Treatment of Androgen in Amago Salmon

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Sex steroids effectively induce sex reversal when given at early stages of gonadal development in teleost fish.^{1,2)} Baker *et al.*³⁾ and Piferrer and Donaldson⁴⁾ successfully masculinized alevins with a single treatment of methyltestosterone (MT) at 20–400 µg/l for 2 h around the time of hatching. This single-treatment method of inducing sex reversal makes it possible to pinpoint not only the time of peak susceptibility to exogenous sex steroids, but also the important period of gonadal sex differentiation. In order to establish the time exogenous androgen most effectively induces sex reversal, genetically controlled all-female alevins of the amago salmon *Oncorhynchus rhodurus* were treated with a single short treatment of MT at various stages of gonadal development.

All-female, eyed eggs were obtained from mating pseudo-male (XX) with normal female (XX). They were transferred from Gifu Prefecture Experimental Fish Station to Teikyo University in Tokyo. They were cultivated in fully aerated water at 8–10°C until use. Fifty alevins each at 3, 8, 11, 14, 17, 22, and 27 days after hatching (DAH) (3–30, Dec. 1990) were immersed for 2 h in water containing 400 µg/l MT (Fig. 1). As a control, 150 fish were immersed for 2 h in 1 l water containing 1 ml ethanol only. Immediately after steroid treatment, autopsies were performed on about 20 individuals from each cohort to assess the effect of the hormone on sex differentiation.

At 100 DAH, 10 individuals were selected from each time cohort, and the number of males was recorded for each. Their gonads were examined histologically. At 200 DAH, male count and histological examination were also done for the remaining 10 fish for each cohort. Normal ovarian differentiation was observed ultrastructurally.

The percentage of sex reversed males in each group is

Fig. 1. Experimental treatment cohorts.

Each arrow indicates the age at which an experimental cohort of 50 fish received a single treatment of methyltestosterone (400 µg/l).

Fig. 2. Percentage of males in each cohort showing susceptibility to methyltestosterone-induced sex reversal.

Letter A indicates a significant difference from 100% females in the control group. (Fisher's exact test, $p < 0.001$). Letter B indicates a significant difference from the sex ratio in the cohort treated at 17 DAH (Fisher's exact test, $0.1 < p < 0.01$).

Fig. 3. An *O. rhodurus* testis at 200 DAH.

This specimen was obtained from the 17 DAH treatment cohort. Efferent ducts (arrows) and spermatogonia are seen, indicating normal testicular differentiation. $\times 115$.

Fig. 4. Electron micrograph of an *O. rhodurus* gonad of control fish at 17 DAH.

Meiotic division of gonadal germ cell (M) is seen. Arrows indicate presumed granulosa cells. G., gonial germ cell. $\times 820$.

shown in Fig. 2. The highest percentage converting to male occurs at 17 DAH. In this group 55% had normal testes with developed efferent ducts and some spermatogonia (Fig. 3). Fish treated at 3–14 and 22 DAH showed lower percentages (10–25%) of conversion to male. No males were found in the cohort treated at 27 DAH or in the control group (100 specimens). These results show that the amago salmon at 17 DAH are more susceptible to exogenous androgen in sex reversal than in the other groups. It should be noted that some fish were also susceptible to MT to change sex from female to male at 3–14 and 22 DAH. This fact indicates that the time of susceptibility to exogenous androgen differs between individuals of this species, though the peak occurs around 17 DAH. Thus, long term treatment covering the susceptible period is necessary to obtain 100% sex reversal, as shown by Usuda.⁵⁾ It is essential to conduct further experiments using pure lines to determine whether this individual difference of susceptibility is genetic or a physiological factor.

Transformation of oogonia to meiotic oocytes in amago salmon gonads provided evidence of ovarian differentiation.⁶⁾ This transformation occurred at 20–25 DAH. Active

mitotic division of oogonia in the cysts, an increase of presumed granulosa cells in the cysts, and other somatic cells characterized female gonads at 17 DAH (Fig. 4). This corresponds to the time of development both in germ cells and in somatic cells just prior to ovarian differentiation.

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