

## Effects of 17 $\beta$ -estradiol (E<sub>2</sub>) on sex-reversal of male Medaka (*Oryzias latipes*), FLF strain, and reproductive potential of the sex-reversed or not reversed individuals

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Dose (E<sub>2</sub>-dissolved in water) and response relationship in the sex-reversal of medaka (*Oryzias latipes*), and sensitive stage in the sex-reversal were investigated by E<sub>2</sub>-exposure within 14 days after the hatch. Sixteen males of the medaka, FLF (female leucophore-free) strain in which genetic gender can be discerned even at the embryonic stages, were exposed to 17 $\beta$ -estradiol (E<sub>2</sub>) for 6 days, from the 3rd day after hatching, at concentrations ranging from 10 to 160 ng/L. Sex reversal rates were checked at the adult stage. The concentration that induced sex reversal in 50% of the male medaka was calculated to be 37 ng/L by a probit regression equation. Vitellogenin concentrations in the livers of adult males increased from 0.67 to 11.4 ng/mg wet weight (mean values) depending on the E<sub>2</sub> exposure concentration. Vitellogenin concentrations in the livers of sex-reversed males (functional females) increased to levels between 1200 and 3600 ng/mg wet weight, irrespective of the E<sub>2</sub> concentration.

The growth stage during which males were most vulnerable to sex reversal was determined by shifting the start of E<sub>2</sub> exposure (200 ng/L, for 2 days) from the 1st day after hatching to the 11th day. Sex reversal rates in cohorts of 15 males exposed to E<sub>2</sub> for 2 days between the 1st and 4th day after hatching was ca. 60 to 70%, and sex reversal rates among those exposed to E<sub>2</sub> between the 3rd and 6th day was around 40%. However, with few exceptions, sex reversal was not observed among the males exposed to E<sub>2</sub> for 2 days during the 6th to 13th day after hatching. The reproduction potential of males, as determined by fertilization rates of eggs produced by mated control females, was more than 90% and was not impaired regardless of whether the males were selected from cohorts in which ca. 70% or 40% sex reversal had occurred. On the other hand, the reproduction potential decreased to ca. 60% in the eggs produced by sex-reversed males (functional females) mated with control males.