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Effects of the Aromatase Inhibitor Fadrozole on Gonadal Development in Coho Salmon, *Oncorhynchus kisutch*

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Abstract

The effects of the aromatase inhibitor fadrozole on oogenesis, spermatogenesis and gonadal development in coho salmon *Oncorhynchus kisutch* were investigated. Both females and males were implanted intraperitoneally with single silicone pellets containing the aromatase inhibitor fadrozole (CGS 16949A) at dosages of 10 $mg \cdot kg^{-1}$, 100 $mg \cdot kg^{-1}$ and 500 $mg \cdot kg^{-1}$, respectively. After 44 d, histological examination showed that females treated at dosages of 100 $mg \cdot kg^{-1}$ and 500 $mg \cdot kg^{-1}$ had gonads with atretic oocytes. Conversely, control females had ovaries filled with synchronously developing oocytes. Both average ova-rian cross-sectional areas and average oocyte diameters in the treated groups were smaller than those in the control. It is suggested that the aromatase inhibitor fadrozole affected oo-genesis and ovarian development in coho salmon. The results did not show any significant differences in spermatogenesis and testicular development between treated and control males, except for the males which received a dosage of 100 $mg \cdot kg^{-1}$, a small number of which had testes which were further developed than those in the control.

Introduction

Aromatase can catalyze the conversion of aromatizable androgens, such as testosterone and androstenedione, to estrogens. Previous investigations have revealed that this process of conversion plays an important part in sexual differentiation and gonadal development in birds (Elbrecht and Smith 1992), reptiles (Lance and Bogart 1992; Desvages *et al.* 1993; Richard-Mercier *et al.* 1995), amphibia (Yu *et al.* 1993) and teleosts (Mayer *et al.* 1991; Piferrer *et al.* 1993; Piferrer *et al.* 1994). Since aromatase activity is particularly high in the brains of teleosts, being 100-1000 times higher than that in other vertebrates (Callard *et al.* 1981), many studies have focused on aromatase distribution and activity in fish brain and pituitary. In Atlantic salmon (*Salmo salar*) male parr, aromatase activity was especially high in hypothalamus and

telencephalon (Andersson *et al.* 1988). In adult goldfish (*Carassius auratus*) and toadfish (*Opsanus tau*), aromatase was concentrated in the pituitary and various forebrain region, especially the hypothalamus/preoptic area (Pasmanik and Callard 1985). In tilapia (*Oreochromis mossambicus*), aromatase was concentrated in the proximal pars distatis (PPD) of the pituitary, being two to five times higher than in rostral pars distalis (RPD) or neurointermediate lobe (NIL) (Callard *et al.* 1988). There also exist some differences in aromatase activity between mature and immature fish and between males and females. It was reported that in vitro formation of estrogens was markedly higher in homogenates of whole brains from mature Atlantic salmon male parr than from immature ones (Andersson *et al.* 1988) and that the female pituitary gland synthesized significantly more estrogens than those of males in the sculpin (*Myoxocephalus octodecimspinosus*) (Olivereau and Callard 1985).

To elucidate the role of aromatase in sexual differentiation and maturation, steroidal and nonsteroidal aromatase inhibitors have been administered to teleosts. Brief treatment with fadrozole caused a small proportion of chromosomally female chinook salmon (*Oncorhynchus tshawytscha*) to develop as normal functional males (Piferrer *et al.* 1994). In Atlantic salmon male parr, three aromatase inhibitors, namely, 1,4,6,-androstatriene-3, 17-dione (ATD), 4-hydroxy-4-androstene-3, 17-dione (4OH) and 4-benzonitrile monohydrochloride (CGS 16949A), influenced gonadal weights in fish sampled in summer; while both ATD and 4OH increased their proportion of maturing males in autumn (Antonopoulou *et al.* 1995). The physiological importance of aromatase in the process of oogenesis and spermatogenesis in teleosts has not been fully elucidated. The purpose of the present study was to evaluate the impact of the aromatase inhibitor fadrozole on gonadal development in female and male coho salmon.

Materials and Methods

One-year-old coho salmon (*Oncorhynchus kisutch*) obtained from Capilano Salmon Hatchery were used in this study. Eighty-six fish, ranging from 8.2-11.0 cm (mean 9.1cm) in fork length and 6.0-10.9 g (mean 7.8 g) in body weight, respectively, were randomly divided into four experimental groups and kept in tanks with mixed flowing creek and well waters. During the experiments, the fish were fed on pellet diets of 1.8-2.5 mm in diameter (Moore Clarke Company) and water temperature varied from 8°C to 10°C. The experiment was carried out on 29 October to 12 December 1996 at the West Vancouver Laboratory aquarium.

Silicone pellets were made by mixing the aromatase inhibitor fadrozole (4-benzonitrile monohydrochloride, CGS 16949 A, Summit, NJ) with silicone elastomere (Factor 11, A-2186). After the mixture hardened , it was cut into 1 x 1 x 5 mm pellets. The tested groups were implanted intraperitoneally with silicone pellets containing fadrozole at dosages of 10 $mg \cdot kg^{-1}$, 100 $mg \cdot kg^{-1}$ and 500 $mg \cdot kg^{-1}$, respectively, and the control group, with silicone pellets containing no fadrozole. The fish were anaesthetized with metomidate hydrochloride (5 $mg \cdot L^{-1}$) prior to implantation and measurement of fork lengths and weights.

At the end of the experimental period (44 d), all fish were dissected and their gonads fixed in 10% buffered formalin phosphate for histology. Samples were dehydrated in ethanol and embedded in paraffin. Sections (5um) were cut and stained in hematoxylin-eosin. As the gonads were too small for accurate weighing, the effects of fadrozole on oogenesis, spermatogenesis and gonadal development were determined by comparing gonadal cross-sectional area, oocyte and spermatocyte diameter and morphological structure in the tested groups with those in the control. In each group, 10 to 13 females were used for the measurement of the ovarian cross-section area and, in each histological section, ten oocyte diameters were measured. T-test was used for statistics in the study.

Results

The effects of fadrozole on oogenesis and ovarian development in females are shown in Figures 1-6. Average ovarian cross-section areas were 14% lower at the low dose, 33% ($p<0.05$) lower at the medium dose and 22% lower at the high dose when compared with the control group (Fig. 1).

Average oocyte diameters were also lower in the treated groups than in the control, although the differences were not significant among them (Fig. 2). Histological examination showed that control females had ovaries filled with normal synchronously developing oocytes (Figs. 3, 5) in the late perinucleolar stage. Conversely, treated females had gonads with some atretic oocytes (Fig. 4), which were being absorbed by phagocytes (Fig. 6). Our results indicate that the aromatase inhibitor fadrozole inhibited oogenesis and ovarian development in the coho salmon.

Fadrozole at a dosage of 100 $mg \cdot kg^{-1}$ had a slight effect on spermatogenesis in male coho salmon. At that level, testicular development in a small number of individuals (29%) was more advanced (spermatocyte, Fig. 7) than those in the control (spermatogonium, Fig. 8). However, in the other two treatment groups, the stage of spermatogenesis was similar to that in the control. There were no significant differences in the average testis cross-sectional area between treated and control groups.

Discussion

The results obtained from the present study demonstrate that the aromatase inhibitor fadrozole inhibits early oocyte development in female coho salmon, thus suggesting that aromatase may play a pivotal role in the process of oogenesis even during the early stage of ovarian development. Afonso *et al.* (1997) reported that fadrozole can significantly reduce 17-estradiol secretion in maturing coho salmon ovarian follicles.There have been several studies on the effect of aromatase inhibitors on sex differentiation in fish, reptiles and birds. Thus, in the alligator (*Alligator* sp.), the steroidal aromatase inhibitor (4OH) caused a moderate disruption of ovarian development while the nonsteroidal aromatase inhibitor (CGS, 16949A) caused inhibition of ovarian development in all treated embryos (Lance and Bogart 1992). In the turtle (*Emys orbicularis*),

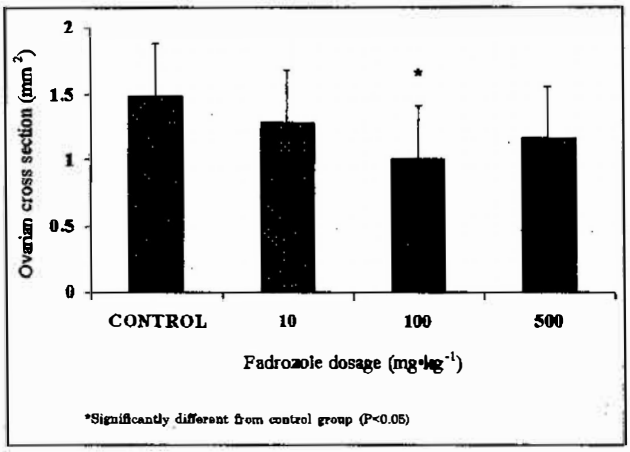

Fig. 1. Effects of fadrozole on ovarian development.

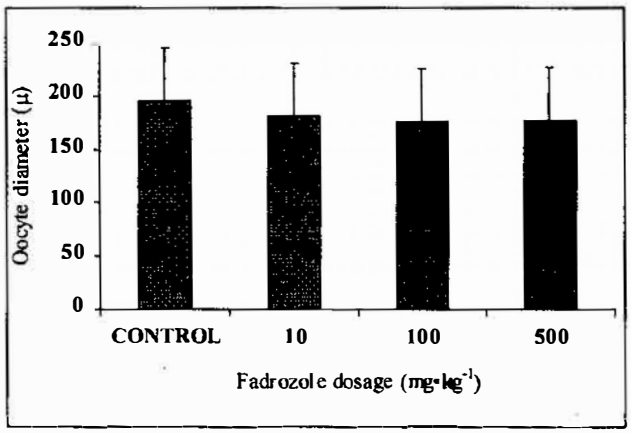

Fig. 2. Effects of fadrozole on oogenesis development.

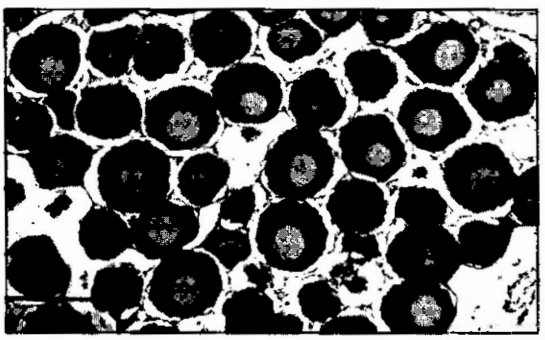

Fig. 3. Control female ovary filled with synchronously developing oocytes, 100x.

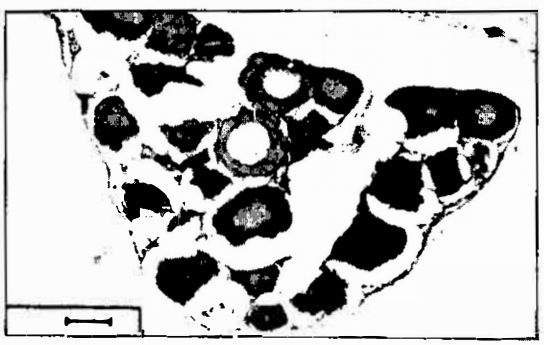

Fig. 4. Treated female ovary with some oocytes in different degrees of degeneration, 100x.

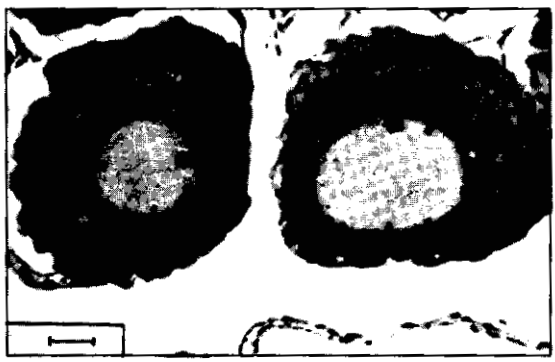

Fig. 5. Normally developing oocyte in the control female ovary, 400x.

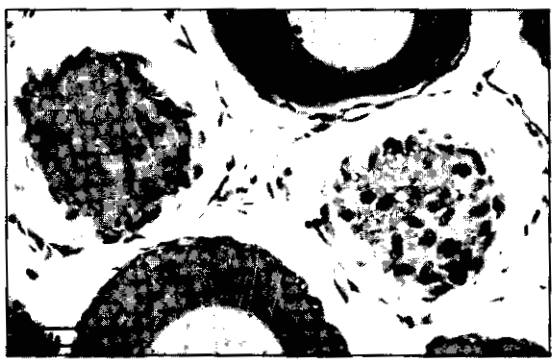

Fig. 6. Regressive oocytes being absorbed by phagocytes in the treated female gonads, 400x.

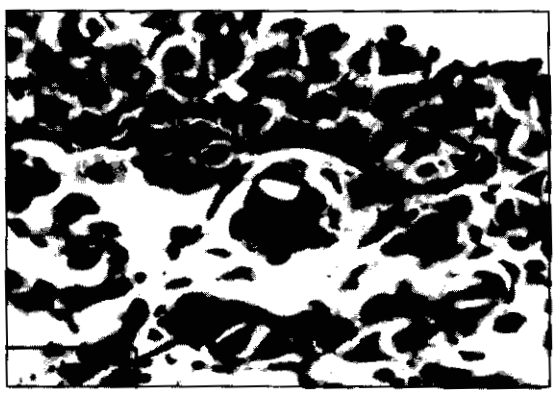

Fig. 7. Spermatocytes (SC), 10-12 um in diameter, 1000x.

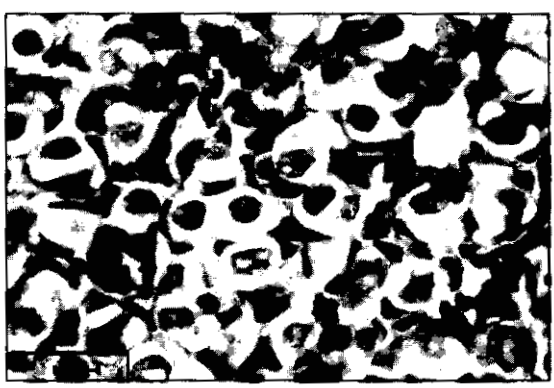

Fig. 8. Spermatogonia (SG), 13-15 um in diameter, 1000x.

the nonsteroidal aromatase inhibitor letrozole induced gonads with different degrees of masculinization at female-producing temperature of 30C (Richard-Mercier *et al.* 1995). A single treatment of chicken embryos with an aromatase inhibitor [5-(p-cyanophenyl)-5,6,7,8-tetrahydroimidazo(1,5-)pyridine hydrochloride] at a stage when their gonads were bipotential caused genetic females to develop a permanent male phenotype (Elbrecht and Smith 1992). Also, in the guppy (*Poecilia reticulata*), a steroidal aromatase inhibitor (4OH) prolonged gestation by inhibiting ovulation (Venkatesh *et al.* 1991). It can be concluded that estrogen appears to be necessary for normal sex differentiation and ovarian development in vertebrates.

However, there has not been any study on fish and the lower vertebrates where investigators have utilized aromatase inhibitors to investigate the role of estrogen during the early stages of oogenesis. Our results, which demonstrate reduced ovarian growth and increased atresia in the presence of fadrozole, suggest that estradiol biosynthesis is essential for the maintenance and continued development of oocytes during early ovarian development. In the present study, we observed only partial inhibition of ovarian development. This may have resulted from the relatively short period between treatment and examination of the fish or from the slow release of the fadrozole from the silicone implants.

Among the three dosage levels, fadrozole at a dosage of 100 $mg \cdot kg^{-1}$ was the most effective. A dosage of 500 $mg \cdot kg^{-1}$ was less effective in blocking oocyte and ovarian development whereas fadrozole at a dosage of 10 $mg \cdot kg^{-1}$ had no significant impact on ovarian development. In chinook salmon, a single 2-h fadrozole immersion during the critical period of gonadal bipotentiality at a dosage of 10 $mg \cdot L^{-1}$ caused 22% of genetic females to develop into males; while a lower dosage of 1 $mg \cdot L^{-1}$ was not effective(Piferrer *et al.* 1994). It can be inferred that the response to fadrozole was dose related. We did not measure aromatase activity or steroidal variations before and after aromatase inhibitor administration in this study. Further investigations on aromatase activity in brains or pituitary and androgen or estrogen levels in plasma are required to demonstrate whether the influence of fadrozole at this stage of ovarian development was at the hypothalamic or ovarian level in coho salmon.

The effects of aromatase inhibitors on spermatogenesis and testicular development in vertebrates vary among inhibitors, animals and dosages. In the coho salmon, an effect of fadrozole on spermatogenesis was only evident at the medium dosage in the present study. In Atlantic salmon male parr, fadrozole-treated fish had a similar gonadal pattern as the control; whereas ATD and 4OH administration increased the proportion of maturating males (Antonopoulou *et al.* 1995). An aromatase inhibitor (4OH) had no apparent effect on testicular development in the alligator (Lance and Bogart 1992). It is possible that the accumulation of certain aromatizable androgens, such as testosterone and androstenedione, has implications for the acceleration of spermatogenesis and the onset of spermiation. In the male coho salmon, fadrozole may have accelerated spermatogenesis and spermiation by blocking feedback inhibition of GtH release. However, it is difficult to explain the lack of effect at the high dose.

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