

A method to Induce Final Maturation and Ovulation in Freshwater-Adapted
Female Chum Salmon, *Oncorhynchus keta*

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A method to induce final maturation and ovulation in freshwater-adapted female chum salmon, *Oncorhynchus keta*

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Abstract

Sexually maturing female salmon (*Oncorhynchus keta*), captured in seashore areas in Yangyang, Gangwon, Korea, in Oct. 2004, were adapted in freshwater over 24 hrs. The experimental fish were injected intraperitoneally with gonadotropin-releasing hormone analog (GnRH_a) (70 ug/kg BW) alone or combined with a dopamine antagonist, pimozide (PIM) (700ug/kg BW). Effects of GnRH_a and PIM on the ovulation, steroid hormone secretion, number of the ovulated eggs and rate of eyed embryos were examined. In the GnRH_a alone or combined groups with PIM, the numbers of the ovulated female increased at 5 and 7 days post-injection and number of the ovulated eggs and the rate of eyed embryos were higher than those of control group. Plasma levels of estradiol-17 β (E2) showed significant decreases in GnRH_a alone and combined treatment with PIM groups at 5th and 7th days after post-injection, whereas the level of 17 α 20 β -dihydroxy-4-progesterone-3-one (DHP) increased. The present study suggests that GnRH_a alone and combined treatment with PIM during oocyte maturation induces ovulation in freshwater-adapted female chum salmon and may apply to an artificial propagation method of salmon seeds without a marked deterioration of the egg quality.

Introduction

In general, the reproductive processes of fish are regulated under the control of hypothalamus-pituitary-gonadal axis. In the axis, gonadotropin-releasing hormone (GnRH) secreted from the hypothalamus of the brain plays an important role in the induction of gonadal development and maturation through the secretion of gonadotropins in the pituitary gland (Ando and Urano, 2005; Kobayashi et al., 1997; Yaron et al., 2003). GnRH stimulates gonadotropin (GTH) release in the pituitary gland and the GTH activates the maturational competence of oocytes via the synthesis and release of the maturation-inducing steroid in the ovary, and in turn, these hormones induce final maturation and ovulation. Furthermore, GnRH-gonadotropin axis was also discovered in the ovary of a fish species, gilthead seabream (*Sparus aurata*) (Wong and Zohar, 2004),

indicating a direct involvement of GnRH in the process of oocyte maturation. Besides GnRH molecules, there are numerous other neurotransmitters and hormones involved in control of GTH production and secretion (Yaron et al., 2003). Among them, dopamine was proven to serve as a GTH-release inhibitory factor (Chang and Peter, 1983) and an anti-dopaminergic drug, pimozide, was highly effective for the stimulation in the natural spawning of fish (Tan-Fermin et al., 1997). During several decades, hormonal manipulations for the induction of final oocyte maturation and spawning have made possible the control of reproductive processes of cultured fish, and have contributed significantly to the sophistication and expansion of the aquaculture industry (Zohar and Mylonas, 2001). In the present study, we studied effects of GnRH and pimozide on the ovulation and the quality of the fertilized eggs in the temporally freshwater-adapted mature chum salmon.

Materials and Methods

1. Fish sampling

Adult female chum salmon, *Oncorhynchus keta* (average body weight (BW): 3.1 ± 0.7 kg, average total length: 65.1 ± 4.2 cm: $n=100$), were collected by netting from seashore areas in Yang-yang, Gangwon, Korea, and transferred to freshwater in an outdoor raceway tank (40.9 X 4.3 X 1.0 m) at East Sea Fisheries Research Institute (Yang-yang, Korea) in October 11, 2004.

2. Hormones

A GnRH analogue, LHRHa (des-Gly¹⁰[D-Ala⁶]-luteinizing hormone releasing hormone, ethylamide, Sigma), and pimozide (PIM, Sigma) were dissolved in physiological saline and mixed with Adjuvant incomplete freund (Difco). The final concentrations of GnRH and PIM were 70 μ g/kg BW and 700 μ g/kg BW, respectively.

3. Experimental protocols

At first, 10 fish were sampled for initial control and the rest animals were randomly divided into three groups ($n=30$, each group) and injected intraperitoneally with GnRH alone, mixed solutions of GnRH and PIM, or vehicle only. At 2nd, 5th, and 7th days after hormone treatments, fish ($n=5-10$) were anesthetized with 2-phenoxy-ethanol and blood samples were obtained from the caudal vasculature with pre-heparinized 20-gauge needles. Then total length, body weight, weight of gonad and liver were measured. For the ovulated fish, the ovulated eggs were fertilized with sperms of chum salmon and the fertilized eggs were maintained at hatchery facilities until the eyed embryos were observed.

4. Hormone measurement

Plasma sex steroids were extracted with Dethyl ether. Plasma estradiol-17 β (E2), testosterone (T) and 17 α 20 β -dihydroxy-4-progesterone-3-one (DHP) were measured by radioimmunoassay (RIA).

Results and Discussion

At 2nd day after post-injection, ovulation was not shown in all groups. At 5th day, number of the ovulated fish increased in GnRH alone group (50%) and GnRH+PIM group (66.7%) than control group (22.3%). At 7th day, both groups received hormone treatment ovulated successfully (92-100%), but not in the control group (16.7%). The rate of eyed embryos was higher in the GnRH alone and GnRH+PIM groups than that of control group.

Plasma levels of E2 in the GnRH-treated two groups were significantly decreased at 5th and 7th days than those of control group, in accordance with a report that decrease of plasma E2 levels occurred in stages of the oocyte maturation and pre-ovulation in amago salmon (*Oncorhynchus rhodurus*)(Young et al. 1983). Plasma T levels dramatically decreased at 5th and 7th days, although significant differences were not observed among the three groups. DHP, a maturation-promoting factor (MPF) of fish, showed significant increases and reached at peak levels at 5th day in GnRH and GnRH+PIM groups. In amago salmon, DHP showed high levels during a stage of oocyte maturation and acted to the oocytes as a final maturation inducing hormone (Nagahama and Achi, 1985).

In summary, the present study revealed that GnRH alone and combined treatment with PIM during oocyte maturation induce ovulation in freshwater-adapted female chum salmon without the deterioration of the egg quality. Thus, it is suggested that the mixed treatment of GnRH and pimozone is a useful method in an artificial propagation of salmon seeds in salmon-hatchery industry.

References

- Ando, H., Urano, A., 2005. Molecular regulation of gonadotropin secretion by gonadotropin-releasing hormone in salmonid fishes. *Zool. Sci.*, 22, 379-389.
- Chang, J.P., Peter, R.E., 1983. Effects of pimozone and Des Gly¹⁰, (D-Ala⁶) luteinizing hormone releasing hormone ethylamide on serum gonadotropin concentrations, germinal vesicle migration and ovulation in female goldfish, *Carassius auratus*. *Gen. Comp. Endocrinol.*, 52, 30-37.
- Kobayashi, M., Amano, M., Kim, M.H., Yoshiura, Y., Sohn, Y.C., Suetake, H., Aida, K., 1997. Gonadotropin-releasing hormone and gonadotropin in goldfish and masu salmon. *Fish Physiol. Biochem.*, 1997, 17, 1-8.
- Nagahama, Y., Adachi, S., 1985. Identification of maturation-inducing steroid in a teleost, the amago salmon (*Oncorhynchus rhodurus*). *Devl. Biol.*, 109, 428-435.

Tan-Fermin, J.D., Pagador, R.R., Chavez, R.C., 1997. LHRHa and pimozide-induced spawning of Asian catfish *Clarias macrocephalus* (Gunther) at different times during an annual reproductive cycle. *Aquaculture*, 148, 323–331.

Wong, T.T., Zohar, Y., 2004. Novel expression of gonadotropin subunit genes in oocytes of the gilthead seabream (*Sparus aurata*). *Endocrinology*, 145, 5210-5220.

Yaron, Z., Gur, G., Melamed, P., Rosenfeld, H., Elizur, A., Levavi-Sivan, B., 2003. Regulation of fish gonadotropins. *Int. Rev. Cytol.* 225, 131-185.

Young, G., Kagawa, H., Nagahama, Y., 1983. Evidence for a decrease in aromatase activity in the ovarian granulosa cells of amago salmon (*Oncorhynchus rhodurus*) associated with final oocyte maturation. *Biol. Reprod.*, 29, 310-315.

Zohar, Y., Mylonas, C.C., 2001. Endocrine manipulations of spawning in cultured fish: from hormones to genes. *Aquaculture*, 197, 99–136.