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Effects of a gonadotropin-releasing hormone analog combined with pimozide on plasma sex steroid hormones, ovulation and egg quality in freshwater-exposed female chum salmon (*Oncorhynchus keta*)

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Abstract

In October 2004 and 2005, sexually maturing chum salmon (*Oncorhynchus keta*) captured by stationary nets in seashore areas of Yang-yang, Gangwon, Korea, were transferred to freshwater and kept over 24 h. To accelerate final maturation and ovulation, the freshwater-exposed female fish were injected intraperitoneally with a single dose of gonadotropin-releasing hormone analog (GnRHa) (70 μg/kg BW) alone or combined with a dopamine antagonist, pimozide (PIM) (700 μg/kg BW). The effects of GnRHa and PIM on the induction of ovulation, the percentages of eyed embryos and hatched alevins were examined together with plasma steroid hormone levels. In the fish treated with GnRHa alone or GnRHa combined with PIM (GnRHa+PIM), the percentage of ovulated females increased on the 5th and 7th days post-injection (38–100%) compared to that of a vehicle only treated group (10–36%) in both 2004 and 2005. By the 7th day of GnRHa and GnRHa+PIM treatment, the percentages of eyed embryos and hatched alevins (79–90% and 50–85%, respectively) were comparable to those of vehicle-treated fish (59–89% and 37–85%). Plasma levels of estradiol-17β (E2) and testosterone exhibited a decreasing pattern with increased duration in freshwater in vehicle-injected fish. In addition, plasma E2 levels were observed to be lower in the GnRHa- and GnRHa+PIM-treated groups than those of a vehicle-treated group over the same time period. The results suggest that temporal freshwater-exposure itself may influence the plasma E2 levels and an additional treatment of GnRHa and PIM acts as a reducer for the lowered E2 levels in freshwater-exposed fish. In contrast, plasma 17α20β-dihydroxy-4-pregnen-3-one levels dramatically increased in the hormone-treated groups throughout the examined period. The present study indicates that administration of GnRHa alone or GnRHa+PIM is effective for induction of sexual maturation and ovulation in freshwater-exposed female chum salmon and dopaminergic inhibition occurs in the maturing salmon. These treatments using hypothalamic hormones for freshwater-exposed female could be applied to the development of a method for artificial propagation of salmon seed without a marked deterioration of the egg quality.

Keywords: GnRHa; Pimozide; Sexual maturation; Ovulation; Freshwater adaptation; Chum salmon

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1. Introduction

Salmon are anadromous fishes, born in the river, grows in the ocean and returns to the natal river for spawning. During their upstream migration in river, females complete their sexual maturation and undergo final oocyte maturation that is hormonally regulated by the hypothalamus–pituitary–gonadal axis (HPG axis) (Urano et al., 1999).

Gonadotropin-releasing hormone (GnRH) secreted from the hypothalamus of the brain plays an important role in the induction of gonadal development and maturation of fish through the secretion of gonadotropins from the pituitary gland (reviewed by Kobayashi et al., 1997; Yaron et al., 2003; Ando and Urano, 2005). GnRH stimulates gonadotropin (GTH) release from the pituitary gland and GTH (especially LH) activates the maturational competence of oocytes via the synthesis and release of the maturation-inducing steroid (MIS) in the gonads. In turn, the MIS induces gonadal maturation and ovulation in salmon (Nagahama et al., 1983; Nagahama and Adachi, 1985). In addition, a GnRH-gonadotropin axis was also discovered in the ovaries of goldfish (Pati and Habibi, 1993, 2002) and the gilthead seabream (*Sparus aurata*) (Wong and Zohar, 2004) as well as rainbow trout (Uzbekova et al., 2001, 2002), indicating a direct involvement of GnRH in the process of oocyte maturation. Besides GnRH molecules, there are certain neurotransmitters and hormones which are negatively involved in control of GTH production and secretion (reviewed by Yaron et al., 2003). Among them, dopamine has been demonstrated to serve as an inhibitory factor to GTH release (Chang and Peter, 1983; Chang et al., 1984).

Over the last few decades, hormonal manipulations to induce final oocyte maturation and spawning have made possible the control of reproduction in cultured fishes and have contributed significantly to the expansion and diversification of the aquaculture industry (Zohar and Mylonas, 2001). The introduction of GnRH analogs has proven to be efficient in inducing maturation and spawning in many marine fish species (Tamaru et al., 1988; Thomas and Boyd, 1988; Zohar, 1988; Slater et al., 1995; Berlinsky et al., 1996; Larsson et al., 1997; Mylonas et al., 1997, 1998). Likewise, an anti-dopaminergic drug, pimozide, has also been found to be highly effective for stimulating the spawning process of fishes mainly in cyprinids and catfishes (Billard et al., 1984; Tan-Fermin et al., 1997).

The timing of sexual maturation and spawning of fish can also be altered by non-hormonal means, such as the manipulation of environmental parameters like water temperature, photoperiod, and freshwater- and/or seawater-adaptation (Takashima and Yamada, 1984; Hirano et al., 1990; Aida, 1991; Pankhurst and Thomas, 1998; Duncan et al., 2000). Interestingly, the percentage of spawned sockeye salmon (*O. nerka*) in a freshwater environment was found to be higher than in seawater during the same time period (Slater et al., 1995). Therefore, the temporal transition from seawater-to-freshwater (SW-to-FW) has been regarded as a convenient and economical means for manipulating maturation and seed production in salmon hatcheries. As mentioned above, accelerating of final maturation and ovulation by hormonal induction combined with an SW-to-FW transition method may shorten the period for maturation and ovulation, and as a result reduce prespawning mortality of salmons in the freshwater environment (Maule et al., 1996; Slater et al., 1995).

The desired outcome of the present work is to establish a practical method to rescue trapped seawater salmon and their maturing eggs. To achieve this outcome required understanding the impacts of GnRHa and pimozide, alone or in combination, on the induction of sexual maturation and ovulation in freshwater-exposed premature chum salmon and the resulting quality of the fertilized eggs. In addition, profiles of plasma sex steroid hormones were monitored during the experiments, to obtain basic information on the in vivo steroidogenic responses using GnRHa alone or in combination with pimozide. This method of reproductive control may be applied for prematuring chum salmon captured by stationary nets in seashore areas and forms the basis for this report.

2. Materials and methods

2.1. Fish sampling

In mid-October, 2004 and 2005, returning female chum salmon in the Korean Peninsula were collected by stationary nets in the seashore region (18.4–19 °C) of Yang-yang, Gangwon, South Korea. The fish were transferred to fresh water in an outdoor raceway tank ($4.3 \times 40.9 \times 1.5$ m) at Yeongdong Inland Fisheries Research Institute, Yang-yang, Gangwon, South Korea and kept over 24 h under natural photoperiod and temperature conditions (14.2–16.0 °C) until the beginning of the experiments.

2.2. Hormone preparation

Female salmon were injected with a GnRH analogue, GnRHa (des-Gly^{10}[D-Ala^{6}]-luteinizing hormone releasing hormone *N*-ethylamide, Sigma-Aldrich, St. Louis,