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Short Communication

Induction of ovulation in endemic *Chalcarburnus chalcoides*, living in the Caspian Sea, using LRH-Aa combined with metoclopramide

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The study was performed in order to try to provoke the gonadotropin wave and ovulation in *Chalcarburnus chacoides* using GnRH analogue, pituitary extract and dopamine antagonist. Different doses and injection protocols were applied using a combination of LRH-Aa, metoclopramide and carp pituitary extract. *C. chalcoides* show wide individual variation and much slower response to different hormonal stimulation. Saline (0.9% Nacl) injected fish were used as a control group and no ovulation occurred in this group. Based on the spawning ratio, fertilization rate and hatching rate, the combination of LRH-Aa 5 µg/kg, carp pituitary extraction 2.7 mg/kg and metoclopramide 2 mg/kg bw doses were found to be more efficient than carp pituitary (4 mg/kg). Following the above procedure it was possible to obtain 80 ± 2% ovulated females in laboratory condition. Fertilization rate was 83 ± 5% and hatching rate 90 ± 3% of fertilized eggs. The survival rate of larvae was 81%. No females ovulated in the treatment group receiving metoclopramide at 10 mg/kg and LRH-Aa alone at 100 µg/kg.

Key words: *Chalcarburnus chalcoides*, spawning, LRH-Aa, metoclopramide, carp pituitary extract.

INTRODUCTION

The *Chalcarburnus chalcoides* is one of the most important fish species in the Caspian Sea. However it is not a commercial fish but is utilized as a food resource by the most commercially valuable fish of Caspian Sea and some species of sturgeons that have persisted relatively unaltered for hundred of millions of years. In spite of the fact that the *C. chalcoides* is considered reproductively active, habitat degradation creates a lot of problems for natural reproduction of this fish. Every year numerous breeders enter the river of southern part of the Caspian Sea but in some rivers their natural reproduction are affected by several threats. The construction of dam, water level regulation and other barriers to fish passage may not only cause depletion of migratory species but also change the upstream habitat or natural reproduction of this fish. Bank side and river bed vegetation removal, canalization and deforestation lead to increased sedimentation and may eventually destroy all habitat attributes for this fish. It is well known that, in such condition (turbid and muddy), ovulation and spawning never occur. Ovulation has to be provoked by natural means and no successful method of artificial reproduction has been reported for this fish as yet.

For artificial spawning, the turning point in inducing breeding of fish by injection of purified pituitary extract started in the 1970s that was widely adopted to different fish species and later by the synthetic GnRH analogues mainly for salmonids. In contrast to salmonids, it was very difficult to induce ovulation in carp by GnRH analogues alone but it is possible by application of specific D2 dopamine receptor antagonists. The methods of inducing ovulation by the use of hypophysation, synthetic hormones together with antidopaminergic drugs showed success in many on fish reproduction (Peter et al., 1988). The objective of present study is to test the potency of synthetic luteinizing hormone-releasing hormone on ovulation and spawning in *C. chalcoides* by using antidopaminergic drugs.

MATERIALS AND METHODS

Experiments were performed during the *C. chalcoides* spawning season in May of 2004 and 2005 at the Department of Aquaculture and Genetics, Ecological Institute of Caspian Sea, Iran. The *C.*

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Table 1. Spawning of *Chalcarburnus chalcoides* after hormone injection.

Group	First injection	Second injection	Spawned
1	Carp pituitary extraction, 0.3 mg	LRH-Aa, 0.1 µg/g bw	-
2	Carp pituitary extraction, 0.3 mg	Metoclopramide, 10 mg/kg bw	-
3	Carp pituitary extraction, 0.3 mg	Carp pituitary extraction, 4 mg/kg bw	8
4	Carp pituitary extraction, 0.3 mg	LRH-Aa 5 µg/kg + carp pituitary extraction 2.7 mg/kg + metoclopramide 2 mg/kg bw	12
5	Vehicle 0.5 ml	Vehicle 0.5 ml	-

For each group, n = 15 with 3 repeat injections at 15 h intervals.

Table 2. Data on ovulation, fertilization and hatching of *C. chalcoides* after hormone injection.

Group	Treatment	Ovulation rate (%)	Total egg (g)	Total spawned	Fertilization rate (%)	Hatching rate (%)
1	LRH-Aa, 0.1 µg/g bw	0^{c}	ND	ND	ND	ND
2	Metoclopramide, 10 mg/kg bw	0^{c}	ND	ND	ND	ND
3	Carp pituitary extraction, 4 mg/kg bw	53.33^{b}	73.63^{b}	134964^{b}	81^{a}	93^{a}
4	LRH-Aa 5 µg/kg + carp pituitary extraction 2.7 mg/kg + metoclopramide 2 mg/kg bw	80^{a}	139.8^{a}	219999^{a}	83^{a}	90^{a}
5	Vehicle 0.5 ml	0^{c}	ND	ND	ND	ND

Means with the same superscript in the same column are not significantly different (P > 0.05).
No data: Fish in this treatment did not spawn and were not included in the statistical analysis.

chalcoides males and females used in this study were caught by gillnet from Tajan River and were disinfected in 3% NaCl solution. Male and female were kept separately in flow-through 2000 L tank in fresh water tanks (18ºC). They were exposed to a simulated natural photoperiod. In total 70 females and 70 males of 3 to 4 years (scale testing) were used and their average body weights were 120 ± 10 and 90 ± 12 g, respectively. Both males and females were starved throughout the experiments. The fish were anaesthetized (MS_{222}, 100 ppm), and 12 h after the pre-injection the fishes were injected by LRH-Aa, (100 µg/kg bw), metoclopramide, (10 mg/kg bw), carp pituitary extraction (4 mg/kg bw) and a combination of them (LRH-Aa 5 µg/kg, carp pituitary extraction 2.7 mg/kg and metoclopramide 2 mg/kg bw). Control females were injected with vehicle (physiological serum 0.9%) at 0.5 ml/kg body weight.

Intramuscular injections were performed by penetration of the dorsal muscles at the base of the dorsal fin. The fish were kept under constant observation in order to detect the ovulation precisely. Ovulation was checked by gently massaging the abdomen 10 - 12 h the second post-injection. For each ovulated female, the total weight of eggs and the weight of fish after stripping were recorded. Eggs from each group were fertilized with a mixture of sperms of two males (0.15 ml of sperm per 1 g of eggs). After fertilization the eggs were washed and placed in Weiss bottles for incubation following a method earlier described for *Rutilus frissii kutum* (Yousefian et al., 2005). When eggs could be stripped easily, males were striped first. After collecting the sperm of 2 males, motility was estimated based on eye observation by eye. Then the ripe female was anaesthetized and hand-stripped. The abdomen was kept dry and the released oocytes were collected in plastic dishes. Oocytes and sperm were mixed with a feather. Freshwater was added and the mixture was gently shaken for 30 s. The GSI was estimated calculating gonad weight/bodyweight × 100 corrected for stripping. During and after incubation, the fertilization rate, the percentage of eyed-stage eggs, and the percentage of hatching were determined for each treatment group. ANOVA analysis was used to test for significant difference among hatching rates.

RESULTS AND DISCUSSION

About 70% of the fish caught in the river were brood fish suitable for induced spawning and the rest were young or spawned fish. In the experiments, nearly all males start spermiation 8 - 10 h after single hypophysation (Table 1). The lowest ovulation success (53.3%) in hormone-treated groups was observed in carp pituitary extraction while injection with combinations of LRH-Aa, metoclopramide and carp pituitary extraction were more effective and the highest ovulation success (80%) was observed (Table 2). Fish injected with LRH-Aa alone, metoclopramide or saline did not ovulate. There was significantly difference in total number of spawned fishes and obtained eggs ($P < 0.05$) between the two groups with ovulated fish. The fertilization rate and hatching rate in groups 3 and 4 were different from each other, and significantly higher than these of other groups ($P < 0.05$).

Out of 15 fish used in treatment with carp pituitary extraction eight fish spawned and 73.63 g eggs were obtained and by a combinant groups 12 fish spawned and 139.8 gram eggs were obtained. After fertilization, the eggs were placed in sisgreen incubators for 24 h at 20ºC.

The major single cause throughout the world of the extinction of population of fish is the destruction of habitat (Mailand, 1987). The fish species are threatened

by a combination of a number of factors, and mainly by habitat alteration. Lowering of the water of the rivers for agriculture or for irrigation purposes, deforestation, water level regulation, dredging, harbors and other physical activities also change the habitat or even destroy it completely. Fish that require the shallow littoral zone for spawning are more likely to be affected by habitat alteration or loss. In this case artificial propagation is the only alternative to rescue the endangered population.

It is well known that synthetic luteinizing hormone-releasing hormone (LH-RH) and its "superactive" analogues such as (LHRH-a) or LRH-Aa stimulate gonadotropin (GtH) secretion in teleosts (Peter, 1983) and stimulate oocyte maturation and ovulation in several finishes species (Chang and Peter, 1983). LH-RH and the analogue LRH-Aa have been shown to be effective, either alone or following a primer injection of salmon GtH, in accelerating the time of oocyte maturation and ovulation of Coho salmon (*Oncorhynchus kisutch*). However, this hypothalamic decapeptide alone did not induce ovulation in common carp (Sokolowska, 1982), gold fish (*Carassius auratus*; Chang and Peter, 1983) and silver carp (*Hypophthalmicthys molitrix*; Lin et al., 1986). An injection of a dopamine antagonist (domperidone or pimozide) to block the effects of dopaminergic inhibitor of LHRH-a-stimulated GtH secretion was required to induce oocyte maturation and ovulation in some cyprinids (Omeljaniuk et al., 1987). According to De Valaming (1983), oocyte enlargement due to hydration occurs in concomitance with maturation, the mechanism of which is said to be hormonally stimulated. In a study, fish injected with LHRH-a or DOM had average oocyte diameter increase similar to the saline-injected fish. This confirms the relative ineffectiveness of LHRH-a or DOM when injected alone in inducing oocyte maturation in bighead carp. Similar to domperidone, metoclopramide is a potent dopamine receptor antagonist. In the present study we found the same results. In ovarian biopsies, from ovaries of female receiving LRH-Aa and metoclopramide alone collected 30 h after treatment, the germinal vesicle was still present in the oocyte. This stimulation shows that these female did not have any progress in oocyte maturation, indicating that LRH-Aa injection alone was not enough to stimulate an adequate increasing in circulating GtH level for induction of ovulation in *C. chalcoides*. However female respond to the dose of LRH-Aa (5 mg/kg bw) when combined with metoclopramide and carp pituitary extraction. This would indicate that in *C. chalcoides* there is a strong dopamine inhibition on GtH secretion.

The fertilization rates were significantly lower in female receiving 4 mg/kg bw carp pituitary extraction ($P < 0.05$). Ovarian biopsies collected 30 h after treatment showed progressive in oocyte maturation with GVBD. These results demonstrated that the carp pituitary extract of 4 mg/kg bw was capable of inducing significant changes in the ovary, but was lower than the minimal effect dose or not proper hormone, to induce complete ovulation and fertilization. Similar results were reported for the low dose of carp pituitary extract treatment in nase (*Chondrostoma nasus*, Szabo et al., 2002) and pike (*Esox lucius*; Billard and Marcel, 1980). Our result indicated that in *C. chalcoides*, there is a strong dopamine inhibitory tone on GtH secretion. This study demonstrated that LRH-Aa 5 µg/kg + carp pituitary extraction 2.7 mg/kg + metoclopramide 2 mg/kg bw treatment was effective and reliable for induction of ovulation in *C. chalcoides* brood fishes.

This investigation has been done to obtain protocols for *C. chalcoides* artificial propagation. The lower combined cost of LHRH-a and metoclopiramide will benefit hatchery operators of this fish. On the other hand, this finding is also important as it is the first attempt to use synthetic hormones in inducing the fish of Caspian Sea for restocking purpose. However it is necessary to conduct more physiological and biological investigations for restocking this fish.

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