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Effect of pituitary extract, ovaprim and combination of human chorionic gonadotropin and metoclopramide on reproductive performance of Caspian shemaya, *Alburnus chalcoides* (Guldenstadt, 1772)

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ABSTRACT

The present study was conducted to examine the effects of three different hormonal treatment types *i.e.,* pituitary extract (Pt), ovaprim (Ov) and human chorionic gonadotropin (HCG) + Metoclopramide (Met) on reproductive performance of Caspian shemaya, *Alburnus chalcoides* (Guldenstadt, 1772). To this end, three experimental treatments and one control group were considered. The experimental groups were administrated with different doses of Pt, Ov, HCG+Met as follows: Pt - 2 mg kg bw (body weight)$^{-1}$, Pt - 3 mg kg bw^{-1}, Pt - 4 mg kg bw^{-1}, Ov - 10 µg kg bw^{-1}, Ov - 20 µg kg bw^{-1}, Ov - 30 µg kg bw^{-1}, HCG+Met - 1000 IU kg bw^{-1}, HCG+Met - 2000 IU kg bw^{-1}, HCG+Met - 3000 IU kg bw^{-1} and also a control group without any hormonal treatment. The highest values of oocyte weight and egg diameter were observed in groups administrated with Ov 10 µg kg bw^{-1} and HCG + Met (2000 IU kg bw^{-1}) respectively. The highest values of absolute fecundity and relative fecundity were recorded for fish administrated with Ov (20 µg kg bw^{-1}). The latency period and hour-degree for final maturation were lower in fish administrated with Ov (10 µg kg bw^{-1}) compared to other experimental groups ($p<0.05$). There were no significant differences between experimental groups in terms of other assayed parameters. The results of the present study demonstrated higher efficiency of ovaprim in improving the reproductive performance of Caspian shemaya.

Keywords**:** *Alburnus chalcoides,* Caspian shemaya, Human chorionic gonadotropin, Metoclopramide, Ovaprim, Pituitary extract

Introduction

The Caspian shemaya *Alburnus chalcoides* (Guldenstadt, 1772) is widely distributed in the Black, Caspian and Aral seas. Populations of the species occur mainly in the western to southern coast of the Caspian Sea and supports local subsistence fishery (Akyurt and Sari, 1991; Balik *et al*., 1996; Bogutskaia, 1997; Tarkan *et al*., 2005). Recently, the fish is considered to be vulnerable to endangered in the south Caspian Basin (Kiabi *et al*., 1999; Naderi and Abdoli, 2004), due to various reasons like damming of the rivers, overfishing during spawning season and deterioration of spawning grounds in the rivers and streams. Reproduction of Caspian shemaya in captive condition could be an appropriate way to produce juveniles for restocking programs and aquaculture goals. Generally, the reproductive cycle can be controlled by either placing the fish in an appropriate environment or by changing the internal regulating factors by injecting hormones or other substances. Artificial induction of fish spawning with hormonal treatments has been used for almost 60 years (Rottmann *et al*., 1991). The spawning induction in carps is usually achieved by the use of human chorionic gonadotropin **(**HCG), synthetic hormones and pituitary extract. Ovaprim (sGnRHa+Domperidone) is widely used for spawning induction of some carp species. The pituitary gland produces and stores gonadotropin hormones (GTH), which play an important role in stimulation of ovulation and spermiation. HCG is purified gonadotropin hormone used for induction of spawning (Rottmann *et al*., 1991). HCG has been increasingly used for induction of spawning in many fish species. Furthermore, HCG acts directly on the gonads (Zohar and Mylonas, 2001). In the present study, we evaluated the effects of three hormonal treatment types *i.e.,* pituitary extract (Pt), Ovaprim (Ov) and HCG+Metoclopramide (Met) at various doses, on the reproductive performance of Caspian shemaya *Alburnus chalcoides*.

Materials and methods

Experimental design

The experiment was conducted at Sefidrood Fishery Research Center (SFRC), Astaneh Ashrafieh, Guillan Province, Iran. Broodstocks of Caspian shemaya were captured from downstream parts of Sardabrood River before onset of spawning season. The broodstocks were transferred to SFRC with proper aeration. Females and males were separated and stocked in different ponds to prevent natural spawning. Thirtysix healthy females (mean weight = 49.5±5.3 g) were distributed in 12 glass aquaria (3 fish per aquarium) as 3 experimental treatments and one control group (without hormonal treatment) with three replicates each. Sex determination was made on the basis of morphologic features. The experimental groups were administrated with different doses of Pt, Ov, HCG+Met at doses of: Pt - 2 mg kg bw^{-1}, Pt - 3 mg kg bw^{-1}, Pt - 4 mg kg bw^{-1}, Ov - 10 µg kg bw^{-1}, Ov - 20 µg kg bw^{-1}, Ov - 30 µg kg bw^{-1}, HCG+Met - 1000 IU kg bw^{-1}, HCG+Met - 2000 IU kg bw^{-1}, HCG+Met - 3000 IU kg bw^{-1} and control group (without any hormonal treatment). Before hormone injection, the fish were anaesthetised using clove oil extract (*Eugenia caryophyllata*) at 30 mg l^{-1} and then hormones were administered by intramuscular injection beneath the dorsal fin. After injection, the fish were placed immediately in the respective aquaria. The fishes were checked for ovulation after first injection at every12 h interval upto ovulation. During the course of the experiment, the water quality parameters in each aquarium was monitored and maintained in normal range of: water temperature - 22ºC, dissolved oxygen - 7.2±0.4 mg l^{-1} and pH - 7.4±0.05. When ovulated, the eggs were fertilised according to Billard *et al.* (1995) using pooled milt samples from three males (39.5±3.5 g) and then incubated separately in 7 l capacity veis incubators until hatching *i.e.,* 3-4 days after fertilisation. In swim-up stage, larvae were transferred to separate Zuger jars (200 l) and fed 10 g dried milk in each Zuger for one week.

Reproductive indices

The reproductive indices were investigated in two stages: (a) after injection (latency period, hour-degree for final maturation) and (b) after ovulation (duration of egg incubation, spawning rate, oocyte weight, egg diameter, number of eggs per gram, absolute fecundity, relative fecundity, fertilisation rate, hatching rate, larvae number and larval survival). These indices were calculated using the following formulae:

Spawning rate = (No. of ovulated fish/Total no. of injected fish) ×100

Fertilisation rate = (No. of fertilised eggs/ Total eggs)× 100

Fertilisation rate was determined under a dissecting loop, 8 h after fertilisation, when the eggs were at the stage of gastrulation (Brommage and Cumalantunga, 1998).

Hatching rate = (No. of viable embryos/Total number of eggs) ×100 (Hanjavanit *et al*., 2008).

Latency period = Time between first injection and ovulation (Drori *et al*., 1994)

Absolute fecundity = Total number of eggs produced per broodfish

Relative fecundity = Total number of eggs produced per broodfish/ body weight (kg)

Survival rate = (Number of live larvae - Number of dead larvae) / Number of live larvae ×100

Statistical analysis

Data normality was tested by Shapiro-Wilk test. Differences between means were analysed using one way analysis of variance (ANOVA) followed by Duncan's new Multiple Range test at minimum significance of $p<0.05$. Differences between means with non-normal distributed data were analysed with Kruskal-Wallis test. Then, Mann-Whitney test was applied to identify which groups were different. All results are presented as means ± standard error of the mean (SEM).

Results and discussion

There were significant differences between experimental groups in terms of ovulation, oocyte weight and egg diameter (Table 1, $p<0.05$). Number of eggs, number of larvae and larval survival did not show significant differences between experimental groups (Table 1, $p>0.05$). The highest values of oocyte weight and egg diameter were observed in fish administrated with Ov (10 µg kg bw^{-1}) and HCG+Met (2000 IU kg bw^{-1}) respectively (Table 1, $p<0.05$). Also, latency period (Fig. 1a), absolute fecundity (Fig. 1b), relative fecundity (Fig. 1c), hour-degree for final maturation (Fig. 1d) and hatching rate (%) (Fig. 1g) showed significant differences between experimental groups ($p<0.05$). In this regard, the highest values of absolute fecundity and relative fecundity were recorded for fish administrated with Ov (20 µg kg bw^{-1}) (Fig. 1b and c, $p<0.05$). The latency period and hour-degree for final maturation were lower in fish administrated with Ov (10 µg kg bw^{-1}) compared to other experimental groups (Fig. 1a and d, $p<0.05$). Also, no significant differences were observed in incubation period (h-degree) between experimental groups (Fig. 1e), fertilisation rate (%) (Fig. 1f) and duration of egg incubation (Fig. 1h) ($p>0.05$).

For inducing artificial reproduction of Caspian shemaya in captivity, broodstocks undergoing stage IV of maturation were required. Therefore, all fish were captured

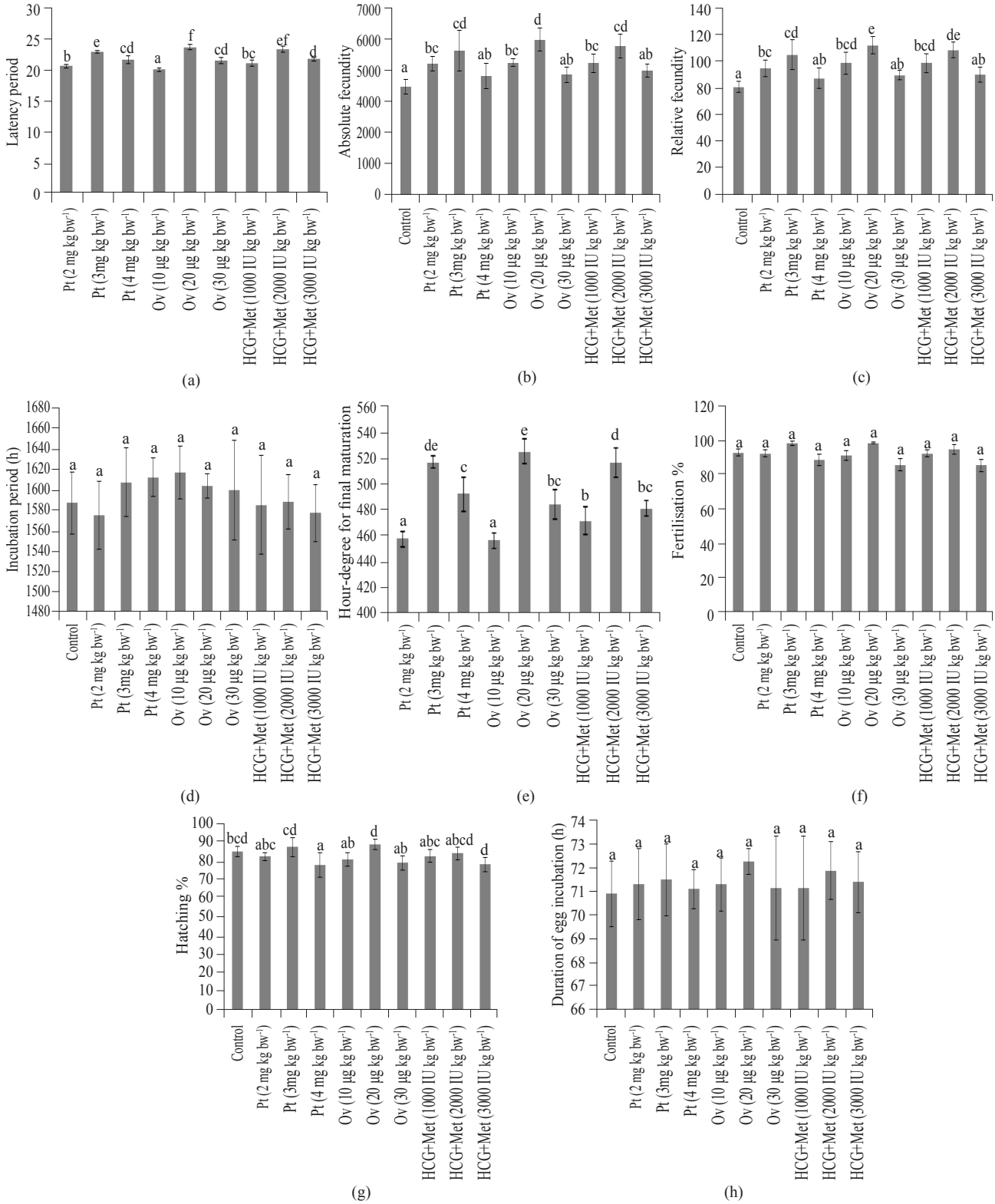

Fig. 1. Comparison of (a) Latency period, (b) Absolute fecundity (eggs per fish), (c) Relative fecundity (eggs per kg fish), (d) Incubation period (h), (e) Hour-degree for final maturation, (f) Fertilisation %, (g) Hatching % and (h) Duration of egg incubation (h) between experimental groups of *A. chalcoides*. Pt: Pituitary extract, Ov: Ovaprim, HCG: Human chorionic gonadotropin, Met: Metoclopramide, IU: International unit. Different letters indicate significant difference ($p<0.05$)

Table 1. Comparison of selected reproductive parameters of *Alburnus chalcoides* between experimental groups

Treatment geoup	Ovulation (%)	Oocyte weight (mg)	Egg number (per g egg)	Egg diameter (mm)	Larvae number	Larval survival (%)
Control	40	4.2±0.14^{a}	982±97.58^{a}	1.15±0.07^{bc}	3455±111.7^{a}	83.5±7.78^{a}
Pt (2 mg kg bw^{-1})	100	5.46±0.31^{d}	919±55.86^{a}	1.1±0.07^{abc}	4080±255.6^{a}	73.2±7.33^{a}
Pt (3mg kg bw^{-1})	100	5.2±0.31^{cd}	970.6±30.5^{a}	1.14±0.09^{bc}	4031.8±172.7^{a}	77±6.44^{a}
Pt (4 mg kg bw^{-1})	80	5.18±0.31^{bcd}	970.25±38.1^{a}	1.13±0.05^{bc}	3839.5±700.4^{a}	81.25±3.59^{a}
Ov (10 µg kg bw^{-1})	100	6.18±0.18^{e}	887.6±103.8^{a}	1.02±0.05^{a}	4740±641.4^{b}	84±4.06^{a}
Ov (20 µg kg bw^{-1})	100	6.1±0.29^{e}	972±34.72^{a}	1.04±0.05^{ab}	5188.2±257.8^{b}	83±6.78^{a}
Ov (30 µg kg bw^{-1})	100	6.1±0.19^{e}	949±51.59^{a}	1.04±0.05^{ab}	4722.6±567.8^{b}	79.4±6.39^{a}
HCG+Met (1000 IU kg bw^{-1})	100	4.76±0.43^{bc}	951.8±21.18^{a}	1.18±0.08^{c}	3473.6±367.8^{a}	77.8±5.85^{a}
HCG+Met (2000 IU kg bw^{-1})	80	4.73±0.33^{b}	969.75±8.96^{a}	1.2±0.08^{c}	3569.5±188.2^{a}	76±4.24^{a}
HCG+Met (3000 IU kg bw^{-1})	80	4.93±0.24^{bc}	962.5±12.72^{a}	1.18±0.1^{c}	3665.8±302.7^{a}	75.6±8.26^{a}

Pt: Pituitary extract, Ov: Ovaprim, HCG: Human chorionic gonadotropin, Met: Metoclopramide, IU: International unit.
Values bearing different superscripts indicate significant difference ($p<0.05$)

from downstream of Sefidrood, since a previous study has showed that almost all the fishes of this area were in stage IV of maturation (Abbasi *et al.,* 1999). According to the results, the shortest latency period was found for fish administrated with Ov (10 µg kg bw^{-1}) and Pt (2 mg kg bw^{-1}) respectively. Nevertheless, there were no significant differences between these treatments with others such as HCG+Met (1000 IU kg bw^{-1}). In fish, duration of latency period is dependent on factors like water temperature, biological characteristics (species, age and weight), hormone type and numbers of injections (Billard, 1990; Yaron, 1995). In our study, the holding conditions for fish were similar and thus the differences between treatments may be associated with the dose of administrated hormone. However, in some studies, the action of Pt in lower levels of hypothalamic-pituitary-gonadal axis (HPG) than gonadotropin releasing hormone (GnRH) was stated as reason for shorter latency period obtained by Pt (Epler, 1986). Spawning rate is one of the good indices for evaluation of hormonal effects on ovulation (Szabo *et al.,* 2002). All administrated fish had higher spawning rate compared to control group with best results in Ov treatments. This results show that Ov could be used for induction of ovulation in addition to HCG and Pt for Caspian shemaya. Several studies have reported the effects of hormonal induction of reproduction on reproductive parameters such as fecundity, egg number, egg diameter, egg weight, larval number and larval survival (Rowland, 1983; Haraldsson *et al*., 1993; Legendre and Oteme, 1995; Mylonas *et al*., 1996; Sahoo *et al*., 2005; DiMaggio *et al*., 2013). Higher egg diameter was obtained in Ov treatments than in HCG, Pt and control groups. Several studies have revealed the positive relationships of egg diameter with egg quality (Zhukinskii and Gosh, 1988). Thus, Ov can improve egg quality in this respect. The values of absolute and relative fecundity in Ov (20 µg kg bw^{-1}), Pt (3 mg kg bw^{-1}) and HCG (2000 IU kg bw^{-1}) groups were higher than in control and other experimental groups which demonstrated that these hormones stimulate ovulation successfully in a dose-dependent pattern.

Results of the present study enhanced our knowledge on reproductive abilities of Caspian shemaya in captive conditions and also helped to compare different methods of artificial induction to determine the best method for controlled spawning in Caspian shemaya.

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