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Survey of Different Hormones on Final Maturation in Shirbut (*Barbus grypus* Heckel, 1843)

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Abstract: One of the main fishes of Karoon river is shirbot, *Barbus grypus* (Heckel, 1843), of Cyprinidae family that is widely distributed in the river systems of the West and Southwest of Iran, especially in Khozestan province, Iran. The objection of this study was to assay the effectivness of a CPE, HCG, LHRHA2, Ovaprim, Ovatide and LHRHa+CPE on spawning success, Latency period, fertilization success and hatching Rate and survival rate%. 56 fish were divided into 7 treatments and injected intramuscullary as follows. Propylene glycol as control, 4 mg kg^{-1} b.w.of CPE, 1000 Iu kg^{-1} b.w.of HCG, 10 µg kg^{-1} b.w.of LRHa, 0.5 ml kg^{-1} b.w. of Ovaprim, 0.5 ml kg^{-1} b.w. of Ovatide, 10 µg+2 mg kg^{-1} b.w.of LHRHa+CPE in double injection 10 h apart. The results showed that LHRHa+CPE combination was 100% spawning success in comparison with HCG, Ovaprim, Ovatide, LHRHA2 and CPE. None of fish were ovulated in the groups of contol, HCG and LHRHA2, while 2/8 fish were ovulated in the group of Ovaprim and 1/8 the group of Ovatide (12.5%). 6/8 fish were ovulated in the group of CPE (75%). Breeding indices were not significantly correlated with females that reposed to induce hormones. Therefore, the results indicated that LHRHA2+CPE combination can be recommended for ovulation of *Barbus grypus* in comparison CPE or alone other hormones.

Key words: Barbus sharpeyi % Spawning % LHRHa % HCG % Ovaprim: Ovatide

INTRODUCTION

Shirbut, Barbus grypus (Heckel, 1843) is a large cyprinid, which occurs along the Euphrates and Tigris Rivers in Iran, Turkey, Syria and Iraq [1] and is abundant and commercially important. One of the main fishes of Karoon river is shirbot, *Barbus grypus*, of Cyprinidae family that widely distributed in the river systems of the West and Southwest of Iran, especially in khozestan province. *B.grypus* lives in fresh and beakish waters, tolerates a wide rang of salinity and temperature and is a euryphage fish migrates from down to upsterm of Karoon river for spawning from the beginning of May [2]. *B.grypus* have economical importance among local people and is an interesting species in commercial aquaculture in Iran.several studies have investigated on *Barbus grypus* but studies about propagation is limited. Its growth, sexual maturity characteristics and reproduction biology have been studied by AL-Hakim *et al*. [3] Elper *et al*. [4] Pyka *et al*. [5] and Szypula, respectively [6]. The spawning period of B.grypus continued approximately from late April to early August. The ovarian type of B.grypus is group synchronous with a capacity for multiple ovulations within a reproductive season [7]. Its reproductive activities, occurred in the northern parts of the regions characterised with sandy or gravel substrate high water current, low temperature and high oxygen content. This condition is not available in Khouzestan plain [8].

Advantages conferred on species with this reproductive strategy include reduction in larval crowding and decreased impact of predation and unfavorable environmental conditions on eggs and larvae [9, 10].

To establish the culture of species, especially cyprinids, that are new for aquaculture, artificial reproduction has been one of the bottlenecks because it has not been possible to reproduce wild cyprinids in hatchery conditions without hormonal stimulation [11-14]. For this reason, many hormonal treatments such as carp pituitary homogenate (CPH), luteinizing hormone releasing hormone (LHRH), HCG, Ovaprim, Ovatide, LHRHA2+CPE have been used for stimulation of gamete maturation in commercial cyprinid culture.

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The aim of the present study was to compare the results obtained after treatment with CPE, LHRH-A2, HCG, Ovatide, Ovaprim and LHRHA2+CPE combination on spawning success in *B. grypus*.

MATERIALS AND METHODS

Fish Stocks and Maintance: The experiments were conducted at south Iran aquculture Research Center, Ahvaz, Khozestan, Iran. Shirbut spawners were collected from earthen ponds of Sosengerd in Iran. 56 female fish weighing 3300-5200 g body weight (b.w.) Females were selected for injections in May based on external characteristics reddish swollen vent and a soft rounded abdomen. Prior to injection, fish were individually weighted and marked by colour cloths on the tail fin and randomly were divided into treatment groups.

Hormones: Fish were divided into seven groups: control group (Propylene glycol) and sex experimental ones. The fish from the experimental groups were treated with two commercial products: Ovaprim contains 20 µg of salmon LHRH analogue (sLHRHa) [d-Arg^{6} Pro^{9} Net-sLHRH] and 10 mg of the dopamine antagonist domperidon in 1 mL propylene glycol [15]. Ovaprim is ready to use in liquid form. Injections were applied intraperitoneally in a single injection at the base of the ventral fin. Fish from the control group were injected with a Propylene glycol solution (0.5 mL kg^{-1}). Luteinizin Hormone-Releasing hormone analogue (Des-Gly10, [D-Ala 6] LH-RH Ethylamide) or LHRHA2 is a peptide that is similar in structure to native lutienzing hormone hormones (LHRH). The LHRHa available on the market is a white powder and is combined with mannite as a filler (made in China).

Before injection fish were anaesthetized with 2-phenoxyethanol (0.5 mL L^{-1}) (Sigma-Aldrich, Germany). Milt from males was collected using plastic 1 mL syringes. Females were checked every 2-3 h between 36 and 48 h post injection. Eggs were stripped into a plastic vessel and were fertilized using the "dry method". Only those samples of milt which showed a motility of more than 70% of spermatozoa were used for fertilization. Three egg samples (100-150 eggs each) from each female were mixed with 0.05 mL of pooled milt taken from at least three males. Oocyte samples from non-ovulated females were taken after the experiment and their maturity stage.

Experiments: Groups of 8 fish were injected I.P. with different preparation: CPE as a group 1(4 mg kg^{-1} b.w) in double injection,Ovaprim and Ovatide 0.5 ml kg^{-1} in a single injection, LHRHa alone 10 µg kg^{-1} in double injection, HCG in double injection. Double injection were done in 10-90% ratio, 10 h apart (Table 1).

After injection,the fish were placed in an indoor fiberglass tank with running water, temperature 23-24°C. The fish were checked for for ovulation after first injection every 10h interval up to ovulation. Although, when ovulation was observed, the eggs were stripped manually and fertilized with milt from at least two males and 250-300 g of fertilized eggs from each female was incubated in vase (7 liter) incubators up to hatching.

Spawning rate (the number of ovulated fish/total number of injection fish) and embryo viability percent (number of viable embryos/total number of eggs×100) were determined [16]. The latency period (the time between the first injection and fish ovulation and working fecundity (the number of stripped eggs/kg b.w.) was calculated according to Drori *et al*. [17] and Billard [18], respectively.

Table 1: The effect of different hormone treatment on spawning success (%),latency period (h), fertilization rate(%), Hatching rate(%) and (%) of Benni, Barbus sharpie

		Dosage						
		----------		Spawning	Latency	Fertilization	Hatching	Survival
Treatment ID	Treatment	1 st	2nd	success (%)	period (%)	rate (%)	rate (%)	rate (%)
Positive control	CPE	0.4mg	3.6mg	75c	22.33±053 a	85.66±1.25a	81.16±0.53a	78.33±0.71a
Negative control		Propylene	glycol	0a	0	0	0	0
T1	HCG (1000) 100Iu	900Iu	0a	0	0	0	0	
T2	LRHa(10)	1µ	9µ	0a	0	0	0	0
T3	LRHa+CPE(10+2)	1+0.2	9+1.8	100c	21.66±0.2a	86.58±0.6a	81.35±0.95a	79.23±0.82a
T4	Ovatide	0.5ml/kg		12.5b	22.5a	84a	83a	79a
T5	Ovaprim	0.5ml/kg		25b	23.55±0.55a	85.00±1a	82.5±1.5a	80.5±0.5a

Mean (± S.E.M.) value with a different letter are significantly different ($p<0.05$)

Fertilization rate was determined under a dissecting loop 8h after fertilization,when were at the stage of gastrulation.

Statistical Analysis: Spawning rate was analysed by the Chi-square test [19]. Differences in latecy period, working fecundity, fertilization rate and hatching rate were analysed by one way analysis of variance (ANOVA) followed by Duncan's new Multiple Range test at minimum significant of $P<0.05$. Results are presented as means±standard error of the mean (S.E.M).

RESULTS

Six of 8 fish ovulated in the CPE group after injection (Table 1). Two of 8 fish ovulated (25%) in the Ovaprim group. The lowest spawning (0%) in the control group, HCG and LHRHa groups were observed. Eight of 8 fish ovulated in the LHRHa2+CPE group after injection (100%).

The latency periods were in the range of 21-24 h after the first injection. The mean latency period was 21.66±0.3 h in LHRHa+CPE group which was lower than all other groups ($P>0.05$). The longest period (23.55±0.5 h) was observed in the Ovaprim group. The mean latency period was 22.5 h in ovatide (T4) (Table 1).

Fertilization rate in treated fish was in the range of 84-86.58% (Table 1) and Ovatide was lowest rate 84 in among groups ($P>0.05$). There was no significant difference in fertilization success among groups ($P>0.05$). The Hatching rate% was in the range 81-83 % and was not showed significantly difference among groups (Table 1). Cotrol group showed lowest Hatching rate ($P<0.5$).

DISCUSSION

In all cases, females from the control groups failed to ovulate and their oocytes did not mature. A similar observation in *B. sharpeyi* and *B. xanthopetrus* was seen that control groups were not ovulated [20, 21], which is similar with reports for other wild cyprinids such as common bream (*Abramis brama* L.), chub (*Leuciscus cephalus* L.), dace (*Leuciscus leuciscus* L.), nase (*Chondrostoma nasus* L.) or ide (*Leuciscus idus* L.) [11, 12, 14, 22, 23]. Hormonal stimulation was necessory to induce ovulation *B. sharpeyi* and *B. xanthopetrus* [20,21] in common carp [23] and other fish species such as northern pike (*Esox lucius* L.) [19] and European catfish (*Silurus glanis* L.) [24]. This indicates that stimulation with environmental conditions alone is not enough to cause final gamete maturation and ovulation under controlled conditions. Application of LHRHA2+CPE in *B.sharpyie* was the best response to spawning induced comparing to other hormones (CPE, Ovaprim, Ovatide, LHRHA2) [20] Also GnRHa, LHRHA2, CPE hormones was used on ovulation induced in *B. xanthopetrus* that was showed CPE hormone is more effective compare to GnRHA and LHRHA2. In the present study, the fish from experimental groups ovulated after hormonal stimulation using CPE, LHRHA2+CPE combination, Ovatide and Ovaprim [21]. The applied doses of both products were the most commonly used in cyprinid controlled reproduction. A similar effect was noted in other cyprinids where these spawning agents were usually very effective in artificial reproduction of wild and cultured fish [19, 24-28]. However, comparison of these data is rather difficult, because different forms of LHRH analogues were used. In the case of *B. sharpeyi* and *B.xanthopetrus*, females wre not stimulated with mammalian LHRHa (alone or with dopamine antagonists) did not ovulate. In contrast, in groups stimulated with Ovaprim (salmon LHRHa with domperidon), lower 50% of females ovulated [19]. In the present study, the percentage of ovulated females stimulated with Ovaprim was much lower than with CPE and LHRHa2+CPE. The percentage of ovulation after using HCG, LHRHA2 was similar (0%). The spawning success, expressed as a percentage of ovulated females and embryos survival in case of shirbut, was high only when Ovaprim was used. LHRH-A has been successfully used for maturation and spawning of various fish including Atlantic salmon [29], seabass and rabbit fish [30] and milkfish [31]. Despite research in our laboratory suggesting that LHRHa about 100% of chines carp ovulate [26] in response to a second dose of 20 µg/kg LHRHa, in this study *B.grypus* had respond lower than CPE treatment. Why the response to hormones was different in *B. grypus* that result was similar to use LHRHa on *B. sharpeyi* and *B.xanthopetrus* The latency period was observed 21.66-23.55 h in treatments that responsed to hormones. The latency period were greater than reported for catfish [29], chines carp [32] common carp [33] and lower than reported for spotted murrel [34] kutum [35], nase [36]. Assessment of effectiveness of hormonal treatments can be done by examinig spawning success, fertilization success, hatching success and survival rate after hormonal treatments.

Fertilization success showed significant differences between LHRHa with CPE, GnRHa treatments suggesting that GnRHa, CPE was similar fertilization success. The hatching rate was no difference in Treatments. The survival rate was not difference (P>0.05).

The type of hormones, administration protocols and gamete acquisition procedures may vary depending on the reproductive biology of each cultured species and a thorough understanding of the endocrine controle of gametogenesis, final maturation and spawning is essential for the appropriate management of the species [37, 38].

In conclusion, this study showed that LHRHa+CPE is an effective and reliable method for induction of ovulation compare to CPE, HCG, LHRHA2, Ovaprim, Ovatide and LHRHa+CPE in shirbut brooodstockand can be very useful for hatchery and broodfish management, spawning and restocking programs.

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