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Effect of GnRHa(D-Ala6,des-Gly10 mGnRHa), LHRH-a(des-Gly10,[D-Ala6] LH-RH Ethylamid) and Carp Pituitary in Artificial Propagation of Gattan, *Barbus xanthopetrus* (heckel, 1843)

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Abstract: The *Barbus xanthopetrus* is a species of the genus Barbus of cyprinidae with local name"Gattan"distributed shadegan and horolazim wetlands in the southwest of Iran. The objection of this study was to assay the effectiveness of a GnRHa, LHRH-a and Carp Pituitary on spawning success, Latency period, working fecundity, fertilization success and Hatching Rate. 32 fish were divided into 4 treatments and injected intramuscullary as follows. 4 mg kg^{-1} b.w.of CPE as positive control, 20 μg kg^{-1}b.w.of GnRHa, 20 μg kg^{-1} b.w.of LHRH-A2, 18μg+2 mg kg^{-1}b.w.of LHRHa in double injection 10 h apart and negative control with distilled water. The results showed that CPE was 87.5% spawning success in comparison with GnRHa, LHRH-a. None of fish were ovulated in the groups of negative control (Distilled water), while 1/8 fish were ovulated in the group of LHRH-a and GnRHa(12.5%).7/8 fish were ovulated in the group of CPE(87%). Therefore, it was concluded that CPE can be effective in comparison with other hormones.

Key words: Spawning · Latency period · Hormone and Fertilization

INTRODUCTION

The *Barbus xanthopetrus* is a species of the genus Barbus of cyprinidae and widely is distributed in the Iraq and Iran [1]. In Iran, this species with local name"Gattan"distributed in karoon and karkhe rivers in the southwest of Iran. It has a synchronous single behaviour spawning on gravelled substrate in kharkhe river in April-May [2]. This is a very valuable commercial fish in the southwest of Iran and a great demand due to its good taste and culinary customs of the local people, although they comprise a small percentage of the catches. The low numbers of these fishes are due to the poor results of natural reproduction and the lack of effective methods of fry production and restocking which could improve the fishery of these species. To restock this valuable species in the wetlands, the Iranian Fisheries Organization (shilat) produced and release up to 3.5 million fry (average weight 1g) in the horolazim wetland annually [3]. Environmental and hormonal manipulation of ovulation in the fish have become of practical importance in the fish farming industry for accelerating or delaying gametogenesis in captive broodstock, spawning may be scheduled to yield fry whenever needed [4]. Use of exogenous hormones is an effective way to induced reproductive maturation and produce fertilized eggs [5]. Originally, culturists utilized carp pituitary (CP) and this is still widely used particularly for the major Indian carps, Chinese carps and the common carp *Cyprinus carpio* [4, 6].

Carp pituitary extract (CPE), the only agent used commonly to induced spawning in gattan, is expensive, not always readily available and with unpredictable potency [7]. An alternative method for gonadotropin realisin hormone agonists(GnRHa), which stimulate secretion of endogenous gonadotropin(GtH) [8]. The success of using GnRHa alone or in combination with DA has been described in several species such as kutum(*Rutilus frisii kutum)* [9], nase (*Chondrostoma nasus*) [10,11], common carp (*Cyprinus carpio*) [12,13], Indian major carps such as rohu (*Labeo rohita*) and mirgal (*Cirrhinus mirigala*) [14-17], catfishes (*Heteropneustes fossilis*) [18]. The objective of induced ovulation is to produce, on demand, a large supply of high quality eggs. Egg quality is assessed by characteristics such as egg fertility and hatching [19]. Hormonal induction of final oocyte maturation and ovulation, however, can result in reduced egg quality [20].

In the present study we investigated the effects of hormone of GnRHa, LHRHa, CPE on spawning success, Latency period, fertilization success , hatching rate in order to develop simple and cost effective method for accelerating and synchronizing ovulation in *B. xanthopetrus*.

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MATERIALS AND METHODS

Fish Stocks and Maintance: The experiments were conducted at south Iran aquculture Research Center, Ahvaz, Khozestan, Iran. Gattan wascaptured from the Horolazim Wetland and maintained in earth pond in January 2003 (water temperature 15-17°C). 32 female fish were with mean weight and length of 3.85±0.45 kg and 64.95±21 cm, respectively. 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 divided into treatment groups.

Hormones: Luteinizin Hormone-Releasing hormone analogue(Des-Gly10, [D-Ala 6] LH-RH Ethylamide) or LHRHa is a peptide that is similar in structure to native lutienzing hormone hormones (LHRH). The LRHa available on the market is a white powder and is combined with mannite as a filler (made in China). TheGnRH agonist D-Ala6, des-Gly1 0 mGnRHa Ethylamide and Dom were supplied as a kit by National Research Institute of Genetic Engineering and Biotechnology, Tehran, Iran.

Experiments: Groups of 8 fish were injected I.P. with different preparation: CPE as a control group (3 mg kg^{-1} b.w), LHRHa alone 20 μg kg^{-1} 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 21-24°C. The fish were checked for for ovulation after first injection every 13 h interval up to ovulation. Although,They can not spawn spontaneously in the tank after hormonal induction so 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 [21]. 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.* [7] and Billard [22], respectively.

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

Statistical Analysis: Spawning rate was analyzed by the Chi-square test [10]. Differences in latency period, working fecundity, fertilization rate and hatching rate were analyzed 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

Non of 8 fish ovulated in the negative groups after injection (Table 1). in the control group, seven out of 8 ovulated (87.5%). one out of 8 ovulated (12.5%) in the GnRHa and LHRHa groups.

The latency periods were in the range of 26.6-27.5 h after the first injection. The mean latency period was 24.42±0.2 h in CPE group was similar than other groups. The longest period (26.6 h) was observed in the LHRHA group (Table 1).

Fertilization rate in treated fish was in the range of 35-77.2% (Table 1) and CPE was highest rate 77.21±3.1 in among groups ($P<0.05$).There was significant difference in fertilization success among groups ($P<0.05$). The Hatching rate% was in the range 76-77.21% and did not show significantly difference among groups (Table 1). The survival rate in CPE,LHRH-a and GnRHa was 85.2±1.29%, 88%,74%, respectivly. Ther was no difference

Table 1: The effect of different hormone treatment on spawning success (%),latency period (h), fertilization Rate(%), Hatching rate(%), Survival Rate of gattan *Barbus xanthopetrus* (Heckel, 1843)

Treatment ID	Positive control	Negative control	T1	T2
Treatment	CPE	Distilled water	LHRHa(20)	GnRHa(20)
Dosage1st	0.4mg	-	2 μg/kg	2 μg/kg
2nd	3.6mg	-	18 μg/kg	18 μg/kg
Spawning success%	87.5 b	0	12.5 a	12.5 a
Latency period	26.88±0.2 a	-	27 a	27.5 a
Fertilization success%	77.21±3.1b	-	35a	65b
Hatching rate%	81.2±1.89a	-	76a	78 a
Survival rate%	85.2±1.29a	-	88a	74a

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

among groups(P>0.05). The survival rate in LHRHA treatment was highest amount(88%),while GnRHa and CPE were 85.2%, 75%, respectively. Non of fish ovulated in the negative control.

DISCUSSION

The necessity of using inducing agents such as CPE, HCG, LHRHa and GnRHa for induction of spawning has been demonstrated in cyprinid fish such as common and Chinese carps [23-26], as well as Indian major carps [25,27,28]. Gattan reproduction in captivity requires hormonal stimulation.To data, there have been no reports of obtaining oocytes from female without it. The spawning success was different among groups (Table 1), with 87.5% spawning success in CPE treatment, a value higher than other treatments. To data showed that GnRHa and LHRHa were able to induced ovulation in 12.5% of the female *B.xanthopetrus* Overall, the results of this study showed that in *B.xanthopetrus* ovulation was not be successfully induced with GnRHa and LHRHa compare CPE. Non of fish ovulated in the negative control. As a result, it is proposed CPE was best treatment for successful spawning induction.

As reported GnRHa is a known spawning inducing agent in Indian major carps, catfish and other carp species [29,24,30,31] and highest ovulation (100%) in Nase, Chondrostoma nasus [11].

LHRH-A has been successfully used for maturation and spawning of various fish including Atlantic salmon [32] , seabass and rabbit fish [33] and milkfish [34]. 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.xanthopetrus* had respond lower than CPE treatment. Why the response to hormones was different in *B. xanthopetrus* to that typically found in Chinese carp is not known. The latency period was observed 26.6-27.5 h in treatments that responsed to hormones. The latency period were greater than reported for catfish [35,36] , common carp [12,13,37], chines carp [38] and lower than reported for spotted murrel [36] kutum [9], nase[11] . 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 [5, 39] .

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

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