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INDUCED SPAWNING OF SILVER CARP, *Hypophthalmichthys molitrix* USING HORMONES/HORMONAL ANALOGUE WITH DOPAMINE ANTAGONISTS

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ABSTRACT: *A study was made to investigate the effects of using carp pituitary extract, human chorionic gonadotropin, luteinizing hormone releasing hormone analogues (Receptal), with or without dopamine antagonists on the spawning performance parameters of silver carp. Results of the current study indicted successful induction of spawning silver carp using different spawning agents. The breeding response and fecundity were comparable among all treatment groups. Moreover, the current experiment clearly indicated that the use of hCG, or mammalian LHRH together with dopamine antagonists was more effective in induction of ovulation and increasing fecundity and hatching rate compared to the other spawning stimulators used in the current study. The results also demonstrated that using dopamine inhibitors potentiate the effect of the hormones used for spawning induction together with reduction of its dose (i.e. dose of carp pituitary extract, human chorionic gonadotropin). Meanwhile, it is well established that domperidone is preferred than metoclopramide as a dopamine antagonists for spawning induction of fish. In view of these results it is clear that not only carp pituitary extract and human chorionic gonadotropin but also the mammalian LHRH analogue (i.e. Receptal) was effective to induce spawning in silver carp. This is important in the view of the fact that mammalian LHRH analogues are available more widely and their price is much more attractive. This would result in cost reduction of induced breeding by using mammalian LHRH analogues in combination with a dopamine antagonist or alone.*

Key words: *Silver carp, induced spawning, human chorionic gonadotropin, luteinizing hormone releasing hormone analogues, dopamine antagonists.*

ORIGINAL ARTICLE

INTRODUCTION

Aquaculture has been known in Egypt since the beginning of written history; tomb friezes date back to 2500 B.C. and illustrate the harvest of tilapia from ponds (Bardach et al, 1972). Modern aquaculture began in the mid-1930s

hormone releasing hormone (LHRH) analogues with dopamine antagonists (e.g., Ovopel, Ovaprim,Dagin, or following the introduction of the common carp at two research farms, from then until the early 1960s; the carp was kept purely for research purposes. With the introduction of modern commercial aquaculture in the late 1970s and early 1980s, Egypt built four carp hatcheries and imported brood stock fish from ex Germany Democratic Republic and Hungary.

Hypophthalmichthys molitrix (Valenciennes, 1844) matures within three years and spawns preferably during mid May to June (Naeem et al., 2005). It is fresh water, omnivorous fish (Williamson and Garvey, 2005). Induced breeding of captive fish may be approached in two ways, hormonal and environmental (Marte, 1989). Artificial reproduction has been one of the bottlenecks because it has not been possible to reproduce wild cyprinids in hatchery conditions without hormonal stimulation (Krejszeff et al., 2008; Kucharczyk et al., 2008; Targońska et al., 2008; Żarski et al., 2009). For this reason, many hormonal treatments such as carp pituitary homogenate (CPE), human chorionic gonadotropin (hCG) or different luteinizing Kabarelin) have been used for stimulation of gamete maturation in commercial cyprinid culture (Kucharczyk et al., 2005, 2008; Brzuska, 2005, 2006; Krejszeff et al., 2008, 2009).

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Hypophysation (use of Carp Pituitary Extract (CPE) to induce ovulation) for spawning induction in fish have been employed in aquaculture since 1930 (Yaron et al., 1999). However, failures have been frequently encountered. This led to the development of new approaches in inducing spawning in cyprinid fishes. Human chorionic gonadotropin (hCG) received some attention as a substitute for pituitary, but has met with little success, excepting in the breeding of silver carp (Chondar, 1985; Chondar, 1990). However, the use of fish pituitaries or human chorionic gonadotropin (hCG) is now limited, owing to inconsistent results, as within 2-3 years, these fish fail to ovulate in response to hCG or carp pituitary extract, making it necessary for fish farmers to continually grow large numbers of female silver carp as brooders (Lin, personal observations, 1986 citted in Kraak et al., 1989).

Accordingly, number of studies conducted in breeding various species of cultured fish in China with LH-RH analogues led to the development of "Linpe method" (Peter et al., 1988). In this approach of induced spawning different LH-RH form and their analogues stimulating endogenous GtH release from the pituitary are used with dopamine receptor antagonist that potentiates the response to the peptide (Zohar and Mylonas, 2001). Currently, emphasis is laid on standardization and reduction of cost of induced breeding by using gonadotropin-releasing hormone (GnRH or LHRH) and their analogues in combination with a dopamine antagonist or alone.

In Egypt the governmental hatcheries are the main source of carp larvae. However the production of these hatcheries become inefficient for meeting the increased demand of silver carp seeds needed for cage and pond culture in the last five years. This, in turn had forced the private sector to make investment in commercial hatcheries for artificial propagation of silver carp.

Therefore, the aim of this study is to investigate the effects of using CPE, hCG and GnRH or LH-RH analogues with or without dopamine antagonists on the spawning performance parameters of silver carp. The possible reduction of carp pituitary dose or other hormone preparations through using dopamine antagonists was also investigated.

MATERIALS AND METHODS

Experimental fish and location

The experiment was conducted on female silver carp *(Hypophthalmichthys molitrix val)* collected from brood stock ponds in carp hatchery complex located at Fowa City, Kafr El-Shaikh governorate, Egypt prior to the breeding season (May, 2007). The brood fish were reared following routine brood husbandry as reported by Jhingran and Pullin (1985).

Experiment protocol

When the temperature reached 20:24°C, fully matured silver carp females (average body weight: 3 to 6kg) and males were selected based on the external secondary sexual characters (Jhingran and Pullin, 1985). Brood fish were randomly divided into eight groups (G_1:G_8) each comprising of four ripe female broods except for groups (G_3,G_7 and G_8) that consisted of 3 fish due to limited number of appropriate ripe fish. The selected males and females were transferred in fiber glass tank filled with water to the hatchery where they were kept undisturbed 3hrs in cycloid steel tanks. The brooders were weighed to estimate the number of pituitary glands needed and amount of hormone and other drugs required for injection.

Preparation of the pituitary gland extracts PCE

The CPE used in this study was prepared from the pituitary glands of adult carps (1-3kg) collected in the pre-spawning season before the beginning of the experiments. Pituitaries were conserved in acetone and were stored as a powder at 4°C. Dry pituitary glands were weighed then grinded in a mortar into powder form. To each g powdered pituitary 10 ml of normal saline (0.7 %) was added. The suspension was centrifuged at 3000 r.p.m then the supernatant was used for fish injection.

Hormone and drugs preparation

Human chorionic gonadotropin hCG (5000 I.U/ml) (Organon Company).

Receptal: each 1 ml contains 0.004 mg Buserelin (Luteinizing Hormone-Releasing Hormone Analogue) Intervet Egypt (S.A.E).

Domperidone (DOM): each 1 ml contains 1mg Domperidone, Glaxo Smithkline Company (GSK).

Metoclopramide: each 1 ml contains 5mg Metoclopramide, SEDICO Company.

Hormone injection

Brood fish were sedated using MS 222 at a dilution of 1:10000 (1g/100 liter water). All brood fish females received two injections of different combinations of CPE, hCG, LHRH analogues and dopamine antagonists (DA) (Table 1). On the other hand brood fish males received one injection of CPE 2h before the time of the second injection of females at a rate of 2 glands/male. Injection was made intramusculary (IM) into the dorsolateral region under the base of the dorsal fin by using hypodermic syringe after cleaning the area with cotton swab soaked in alcohol.

Stripping

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Six hours after final injection the bottom of the fish tanks was checked regularly for the presence of released eggs, which indicated the approximate spawning time. Once eggs were identified in the tank (Drori et al., 1994; Brzuska, 2004), fish were examined every 30 minutes and ovulated fish were anesthetized and stripped by slight pressure on abdominal region. The males were caught from the cycloid steel fish tank and milt was squeezed-out of the males into fertilizing solution. Ovulated eggs were manually stripped from the female and collected in dry plastic vessels. All eggs were immediately weighed and samples were taken to determine the number of eggs per gram and the total number of eggs per female. The eggs of each fish were weighed and fertilized by a mixture of sperm collected from two hormonally induced males. Continuous stirring was performed while adding fertilizing solution every 3 minutes until complete fertilization occur within 15 minute.

Table 1 - Hormonal doses used in different brood fish silver carp groups for spawning induction

Treatment	Dose	Number of fish
G_1: (CPE)[1]	• 1st dose (females) 3mg CPE /female. • 2nd dose 8hrs later 3.5mg CPE/female	4
G_2 : (CPE)[1] + (DOM)[2]	• 1st dose 3mg CPE /female + 1mg DOM. • 2nd dose 8hrs later 1.5 mg CPE /kg +0.5mg DOM	4
G_3: (CPE)[1] + (MET)[3]	• 1st dose 3mg CPE /female + 5mg MET • 2nd dose 8 hrs later 1.5mg CPE /kg + 2.5mg MET	3
G_4: (hCG)[4]	• 1st dose 250:300 IU/Kg. • 2nd dose 12 hr later 1500:1800 IU/Kg.	4
G_5: (hCG)[4]+ (DOM)[2]	• 1st dose 250:300 IU hCG/kg + 1mg DOM • 2nd dose 750:800 IU /kg hCG+ 0.5mg DOM	4
G_6: (hCG)[4]+ (MET)[3]	• 1st dose 250:300 IU/Kg + 5mg MET • 2nd dose 750:800 IU/hCG+ 2.5mg MET	4
G_7:Buserelin (LHRHa)[5]	• 1st dose 1ml (0.004mg) Buserelin /kg • 2nd dose 12h later 1ml Buserelin/kg	3
G_8:Buserelin (LHRHa)[5] + (DOM)[2]	• 1st dose 1ml Buserelin /kg + 1mg DOM • 2nd dose 12h later 1ml Buserelin/Kg +0.5mg DOM	3

1. Carp pituitary extract; 2. Domperidone; 3. Metoclopramide; 4. Human chorionic gonadotropin; 5. Luteinizing hormone releasing hormone analogue.

Incubation of fertilized eggs

In Fowa Governmental Artificial Hatchery the German System is used (System of Glass Aquaria). At first glass aquaria (150 X 50 X 50 cm^3) were treated with formalin diluted at the rate of 1: 10000 before incubation of eggs. Thereafter, the aquaria were supplied with water. Eggs were incubated in aquaria at the rate of 2 kg/aquarium. The water volume in each aquarium was about 235.5 liter with a water flow rate maintained at 1 liter /minute. There was an air pump tube under the surface of water in order to help in stirring of eggs. Water temperature during egg incubation was 20 - 24°C. Eggs for each aquarium were treated with formalin diluted at the rate of 1: 10000 every four hours

The fertilization and hatching successes were determined according to Rothbard (1981). Spawning success (the number of ovulated fish/total number of treated fish), the latency period (the time between treatment and ovulation) and practical fecundity (number of stripped egg kg^{-1} body weight before stripping) were calculated according to Drori et al. (1994) and Szabó et al. (2000).

Statistical analysis

One-way analysis of variance (ANOVA) was applied ~~used~~ using (Statistical analysis System (SAS) software (SAS Institute Cary, North Carolina, USA, 2004) to fulfill the requirement of the following statistical model:

$X_{ijk} = \mu + T_i + R_j + e_{ijk}$

X_{ijk} = observed value

μ = population mean

T_i = Effect of treatment i,

R_j = Effect of replicate j

e_{ijk} = random error

RESULTS

The results of the different trials on the induced spawning of silver carp with CPE, CPE+DOM, CPE+MET, hCG, hCG+DOM, hCG+MET, buserelin and buserelin+DOM are presented in Table 2.

The brood fish females of different treatment groups spawned completely, as indicated by the breeding response. However, no significant differences ($P > 0.05$) observed in the female fecundity among all of the treated

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brood fish groups. On the contrary, the female quantity of spawn/kg of female differed significantly ($P < 0.05$) among different treatment groups. Brood fish of G_6 and G_5 showed the highest level of fecundity however they were not significantly differed ($P > 0.05$) from G_1 G_2, G_3, G_4 and G_7. On the other hand, the fecundity of G_8 brood fish was significantly ($P < 0.05$) lower than the other treated brood fish groups. Similarly, G_5 and G_6 brood fish had the highest significant ($P < 0.05$) fertilization rate and hatching rate, whereas, G_1 brood fish was the lowest. Meanwhile the recorded fertilization rate of G_5, G_6 brood fish and hatching rate of G_5 brood fish was not significantly differed ($P > 0.05$) from those recorded for G_8 brood fish. Additionally, G_7 brood fish and G_8 brood fish had significantly higher latency period ($P < 0.05$) which was not significantly differed among the other brood fish groups.

Table 2 - (Means±SD) for the effect of different treatments on spawning parameters studied in silver carp.

Treatment	no. of fish spawned	Broodstock weight(kg)	Quantity of spawn (kg) produced / female	Total No. of eggs/kg female B.Wt	Fertilization rate (%)	Hatching rate (%)	Latency period (hours)
G_1: (CPE)[1]	4/4	4.50±1.29[a]	0.45±0.13[ab]	36872±11254[a]	86.50±1.29[d]	83.50±1.29[d]	7.50±0.58[c]
G_2 : (CPE)[1] + (DOM)[2]	4/4	5.00±1.47[a]	0.48±0.13[ab]	35602±13058[a]	89.50±1.00[c]	86.50±1.00[c]	7.50±0.58[c]
G_3: (CPE)[1] + (MET)[3]	3/3	4.83±0.58[a]	0.41±0.05[ab]	30008±3353[a]	90.66±1.15[bc]	87.00±1.00[c]	7.67±0.58[c]
G_4: (hCG)[4]	4/4	4.63±1.38[a]	0.45±0.13[ab]	36166±11815[a]	89.50±1.00[c]	86.25±1.26[c]	7.50±0.58[c]
G_5: (hCG)[4]+ (DOM)[2]	4/4	5.00±1.83[a]	0.50±0.18[a]	31004±8261[a]	92.50±0.58[a]	88.75±0.50[ab]	7.50±0.58[c]
G_6: (hCG)[4]+ (MET)[3]	4/4	5.38±0.48[a]	0.53±0.05[a]	33892±2918[a]	92.75±0.50[a]	89.25±0.96[a]	7.25±0.50[c]
G_7:Buserelin (LHRHa)[5]	3/3	4.00±0.5[a]	0.35±0.05[ab]	30667±3834[a]	90.00±0.00[bc]	86.00±1.00[c]	11.00±1.00[a]
G_8:Buserelin (LHRHa)[5] + (DOM)[2]	3/3	3.33±1.04[a]	0.32±0.08[b]	34798±9812[a]	91.33±1.15[ab]	87.33±0.58[bc]	10.00±0.00[b]

Means within the same column carrying different letters are significantly different at ($P < 0.05$). 1. Carp pituitary extract; 2. Domperidone; 3. Metoclopramide; 4. Human chorionic gonadotropin; 5. Luteinizing hormone releasing hormone analogue.

DISCUSSION

Results of the current study indicated successful induction of spawning of silver carp using different spawning agents; carp pituitary extract (CPE), human chorionic gonadotropin (hCG) or luteinizing hormone releasing hormone (LHRH) analogues with or without dopamine antagonist. These results are in agreements with the results obtained by several studies (Kucharczyk et al., 2005, 2008; Brzuska, 2006; Basavaraja et al., 2007; Krejszeff et al., 2008, 2009; Vazirzadeh et al. 2011). The breeding response and fecundity were comparable among all treatment groups. Similarly, Brzuska, (1999) failed to detect significant difference between LHRHa plus dopamine antagonist (Pimozide) or CPE silver carp treated fish in spawning index. Additionally, Brzuska and Bialowąs (2002) showed no significant effects of Ovopel (mammalian GnRHa+dopamine antagonist, Metoclopramide) or CPE on egg weights. Nevertheless, in another study, Brzuska (2003), however, found high statistically significant values of egg weight for fish treated with three different treatments: CPE, Ovopel and CPE plus Ovopel.

On the other hand, the addition of dopamine antagonists successfully increased fertilization rate and hatching rate on hCG+MET, hCG +DOM and Buserelin (LHRH) +DOM treated brood fish groups. *Aizen et al. (2005)* indicated that, the addition of some additives to hCG or CPE as a dopamine antagonist (DOM + GnRHa) causes the stimulator was more potent in inducing ovulation and spawning as compared to GnRHa alone or dopamine antagonist alone and this attributed to Dopaminergic inhibition is a major barrier along the reproductive axis that arrests spontaneous spawning. Similarly, simultaneous injection of pimozide (10 mg/kg) plus LHRH-A (100 pg/kg) caused a high rate of ovulation and the fertility of ovulated eggs (75% > was similar to that of the hCG spawned fish (Lin et al., 1987).

The higher spawning results due to using hCG+DOM may be attributed to that, Human Choronic Gonadotropin (hCG) is the most common purified Gonadotropin hormone used for induced spawning. hCG by-pass the brain–pituitary link, acting directly on the ovaries and testes (Rottmann, et al.,1991). Meanwhile, it is well established that DOM is the preferred DA for spawning induction of fish because DOM does not cross the blood-brain barrier (Omeljaniuk et al., 1987), and DOM can cause a long-lasting, dose-dependent depletion of dopamine in the pituitary (Sloley et al., 1991), which in turn my explain the success of spawning induction in this study with reduced doses of all the hormones used in combination with dopamine antagonists.

Concerning the latency period, all silver carp brood fish began spawning 7-12h after hormones injection with or without DA injection. These results are in agreement with the results obtained by other several studies irrespective of induced spawning of many cyprinids including silver carp (Ngamvongchon et al., 1988; Peter et al., 1988; Brzuska, 2006; Basavaraja et al., 2007; Makeyeva *et al*., 1996; Kłodzińska and Kozłowski 1991; Vazirzadeh

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et al., 2011). Additionally, The fact that the silver carp females yield eggs in a short time interval (in case of both LHRH-a and other hormones used in the current study) is very important on account of the short period in which the optimum spawning occurs in herbivorous fish at temperatures between 20 °C and 26 °C (Zonnenveld 1984; and Brzuska, 1999).

However, it seems to be worth stressing that the females of silver carp began spawning more than 9 h after the LHRH-a and pimozide injection at a temperature of 20-24°C. In the Linpe method (Peter et al., 1988), an equal latency of 8–12 h was recorded for these species of herbivorous fish at the temperature of 18–30°C, irrespective of the application of mammalian or salmon analogues. On the contrary, Ngamvongchon et al. (1988) and Makeyeva et al., (1996), recorded a latency time of more than 20h upon using LHRH-a analogue as ovulation stimulator in silver carp.

Conclusion

The results of this experiment indicated that injection of silver carp with using hCG, or mammalian LHRH together with dopamine inhibitors was more effective in induction of ovulation and increasing fecundity and hatching rate compared to the other spawning stimulators used in the current study. The results also demonstrated that using dopamine inhibitors potentiate the effect of the hormones used for spawning induction together with reduction of its dose (i.e. dose of carp pituitary extract, human chorionic gonadotropin). Meanwhile, it is well established that domperidone is preferred than metoclopramide as a dopamine antagonists for spawning induction of fish. In view of these results it was clear that not only carp pituitary extract and human chorionic gonadotropin but also the mammalian LHRH analogue (i.e. Receptal) was effective to induce spawning in silver carp. This is important in the view of the fact that mammalian analogues are available more widely and their price is much more attractive. This would result in cost reduction of induced breeding by using mammalian LHRH analogues in combination with a dopamine antagonist or alone

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