Induction of spawning in common carp *Cyprinus carpio*, using pituitary extract and GnRH analogue in combination with Domperidone

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Abstract

The effectiveness of the first Iranian made gonadotropin releasing hormone analogue, [D-Ala^6 des-Gly^{10}] GnRH ethylamide, alone or in combination with domperidone, a dopamine antagonist on spawning rate, latency period, working fecundity and embryo viability in common carp, Cyprinus carpio, was investigated. Fifty two fish were divided into 5 groups and treated intrapretoneally as follows: 3 mg/Kg b.w. of carp pituitary extract (C.P.E.) as a positive control, GnRHa alone, 10 µg/Kg b.w.. or in combination with domperidone, 5 mg/Kg b.w. in a single or double injections 7h apart. A group was treated with propylene glycol 0.2 ml/Kg b.w. alone and considered as control. No female ovulated in groups receiving either propylene glycol or 10 µg/Kg b.w. of GnRHa alone. The spawning rate was higher in female GnRHa+domperidone (10 µg/Kg b.w..+5 mg/Kg) in double injections (11 out of 12) as compared to fish which injected either with C.P.E (7 out of 16) or GnRHa + domperidone in a single injection (3 out of 12)($P<0.05$). The mean working fecundity, was significantly higher for fish receiving GnRHa+domperidone in single (126214 ± 24315) or double injections (145600 ± 27113) compared to C.P.E treated group (52435 ± 1224) ($P<0.05$). There were no significant differences for latency period or embryo viability among the groups.

Keywords: *spawning induction, carp pituitary extract, GnRH analogue, domperidone.*

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INTRODUCTION

Modern aquaculture aims to provide a low cost, high quality products according to market demand. Supplying an ondemand consumer products require a reliable and constant production system, which begins with constant supply of eggs and larvae. In a number of cultured fish such as salmonids, the female ovulate spontaneously, but it is necessary for many others to control ovulation and spawning time to enhance reproduction performance.

Induced spawning in common carp, *C. carpio*, is currently carried out in Iran by the hypophyseal approach utilizing carp pituitary extract (C.P.E) which is expensive, not always readily available and with unpredictable activity (Drori *et al.*, 1994). The success of this method is quite variable and average percentage of ovulated female carp reaches only about 60-70% in fish farms around the world (Weil *et al.*, 1986). Furthermore, there is a possibility that pathogen may be present in the donor fish and can be passed on to recipient fish (Zohar, 1989). With respect to these factors, searching for an alternative approach to spawning induction in cultured fish have started. The latest approach is the stimulation of spawning by a synthetic superactive analogue, to release the endogenous gonadotropine (GtH) from the pituitary of treated fish. One of the most effective analogues is [D-Ala^6 des-Gly^{10}] *GnRH* ethylamide (Zohar, 1989).

To facilitate the GtH releasing activity of *GnRHa*, especially in cyprinids, it is necessary to combine it

with a dopamine receptor antagonist such as domperidone, pimozide or metoclopramide (Peter *et al.,* 1988).

Induction of spawning in fish using a superactive *GnRH* analogue together with one of these dopamine antagonists is known as the Linpe method (Peter *et al*., 1988) which is now used in many parts of the world. The success of using *GnRHa* alone or in combination with dopamine antagonist in spawning induction of various fish has been reviewed by Zohar, 1989; Peter *et al.,* 1988; Yaron, 1995; Zohar and Mylonas, 2001 and Szab *et al.*, 2002. The induction of spawning in cultured cyprinids in Iran including common carp (Ghezel, 1993) has been reported using Receptal (*GnRHa*) combined with metoclopramide. The effectiveness of the Iranian made *GnRHa* for induction of spawning (ovulation and spermiation) in rainbow trout i.e. *Oncorhynchus mykiss,* was also evaluated recently (Dorafshan *et al.*, 2002; Paykan heyrati *et al.,* 2002).

The objectives of the present study were: a) To examine the effects of *GnRHa* alone or combined with domperidone on induction of spawning in common carp. b) To compare two methods of induction of spawning, *i.e.* hypophyseal and Linpe methods, with respect to the spawning rate (the number of ovulated fish/number of injected female), latency period (the time between treatment and ovulation), working fecundity (the number of stripped eggs/Kg b.w.) and embryo viability (the number of viable embryos divided by total number of eggs×100).

MATERIALS AND METHODS

Stock: Spawning experiments were conducted on 2-3 years old common carp females, *C. carpio,* at Shahid Maleki fish farm, Ahwas, Iran, during March 2002. Fifty two female fish weighing 2.8-4 kg b.w. were selected from earthen ponds for ripeness. This selection was based on the softness of their abdomens as pointed out by Weil *et al.* (1986). Fishes were then transferred into indoor concrete tanks with running water of 20-22°C. Prior to injections, fish were anesthetized in 100 ppm MS222 bath, individually weighed and marked by placing visible tags on dorsal fin, randomly divided into treatment groups.

Hormone preparation: A *GnRH* analogue (D-Ala^{6} Des Gly^{10}) *GnRHa* ethylamide and dopamine receptor antagonist, domperidone were supplied by NRCGEB, diluted in 40% propylene glycol to achieve a concentration of 10 µg of *GnRHa* with or without domperidone 5 mg per kg b.w. at a final injection volume of 0.2 ml/kg b.w. Three mg pituitary glands per kg. b.w. of broodstock was used for induction of spawning in solution form (0.7% saline) according to Billard (1990).

Experiments: Fifty two fish were anaesthetized and injected intraperitoneally (i.p.) with diffrent preparations as follows: vehicle, propylene glycol as a negative control (n=6), carp pituitary extract (C.P.E.) as positive control (n=16), GnRHa alone (n=6), *GnRHa* +domperidone in a single (n=12) or double injection (n=12) in groups 1-5, respectively (Table1). Females were checked for ovulation, every 30-45 min. 9 h after final the injection. When ovulation was observed, the eggs were stripped and batches of approximately 200g were collected from each individual female and fertilized with milt from at least two males (Szabo *et al*., 2002). Batches of fertilized eggs were incubated separately at 20-22°C.

Spawning rate (the number of ovulated fish/total number of injected fish) and embryo viability percentage (number of viable embryos/total number of eggs ×100) were determined according to Kulikovsky *et al*. (1996). The latency period (the mean time between treatment and ovulation) and working fecundity (the number of stripped eggs/kg b.w.) were calculated

Table 1. Dosage and hormonal preparations used for inducing spawning in Common carp, *C. carpio*.

Groups	Treatment	Fish No.	Injection dosage /kg b. w.		Time interval
			First	Second	h.
1.	Propylene glycol (vehicle)	6	0.2 ml	-	-
2.	Carp pituitary extract (C.P.E)	16	0.3 mg	2.7-3 mg	7
3.	GnRH	6	20 µg	-	-
4.	GnRH+ domperidone	18	10µg+ 5 mg	-	-
5.	GnRH+domperidone	12	1µg+ 0.5 mg	9µg+4.5 mg	7

Table 2. Spawning rate and latency period in common carp, *C. carpio* following hypophyseal treatment or GnRH alone or GnRH combined with domperidone.

Group	Treatment	Spawning rate	latency period (h)
1.	Vehicle	0/6 [a]	-
2.	Carp pituitary extract (C.P.E)	7/16[b]	10-12[n.s]
3.	GnRH	0/6[a]	-
4.	GnRH + domperidone (single injection)	3/12[b]	14-16[n.s]
5.	GnRH + domperidone.(double injection)	11/12[c]	10-14[n.s.]

Groups designated by the same letters are not significantly different (P>0.05).
n.s.: no significant.

Table 3. Working fecundity (stripped egg No/kg b.w.) and embryo viability (%) in common carp, *C. carpio*, following hypophyseal, GnRH alone, or GnRH combined with domperidone treatment.

Group	Treatment	Working fecundity	Embryo viability (%)
1	Vehicle	0[a]	-----
2	C.P.E.	52435±1224[b]	64.3±5.6[n.s.]
3	GnRH	0[a]	------
4	GnRH+domperidone (single injection)	126214±24315[c]	57.8±6.6[n.s.]
5	GnRH+domperidone(double injection)	145600±27113[c]	62.4±4.8 [n.s.]

Groups designated by the same letter are not significantly different (P>0.05).
n.s.: no significant.

according to Drori *et al*., 1994 and Billard,1990, respectively.

Statistical analysis: Spawning rate was analyzed by chi-square test (Szabo *et al*., 2002) the differences in latency period, working fecundity and embryo viability data were analyzed by one way analysis of variance (ANOVA) at minimum significant of P<0.05. Results are presented as means±S.E.M (Kulikovsky *et al*., 1996).

RESULTS

The fish used in this study were in pre-spawning stage. The results of the effects of hormonal treatment on spawning rate and latency period are summarized in table 2. No ovulation was observed in groups receiving either vehicle (propylene glycol) or *GnRHa* alone (groups 1 and 3, respectively). Seven out of sixteen (7/16) fish ovulated in group 2 receiving C.P.E. as the positive control (approx. 43%). The number of ovulated fish in groups 4 and 5 which received *GnRHa* +domperidone as single or double injections were three and eleven out of twelve respectively. There was no significant difference in spawning rate between groups 1 and 3 (receiving vehicle or *GnRHa* alone) and groups 2 and 4 (receiving C.P.E or *GnRHa*+domperidone in single injection). However the spawning rate in group 5 which received *GnRHa*+domperidone in 2 injections protocol 7 h. apart, was significantly higher than all other groups (P<0.05). Data on latency period are also shown in table 2.

Ovulation in females was detected 10-16 h after treatment, the latency period were measured as 10-12, 14-16 and 10-14 h in groups receiving C.P.E. and single or double injection *GnRHa*+domperidone respectively (Table 2). Although the latency periods were relatively higher in *GnRHa*+domperidone injected groups as compared with C.P.E. treated fish, but there were no significant differences between the groups.

The mean working fecundity in ovulated fish is shown in table 3. The mean working fecundity in treated fish with single or double injection of *GnRHa* +domperidone were 126216 ± 24315 and 145600 ± 27113, respectively, and significantly higher than C.P.E. treated fish, 54435 ± 1224 (P<0.05). The per-

centages of embryo viability are also shown in table 3, when was 64.3 ± 5.6, 57.8 ± 6.6 and 62.4 ± 4.8 % in groups receiving C.P.E., *GnRHa*+domperidone in single and double injected groups respectively. Although the mean embryo viability was lower in group treated with single injection of *GnRHa*+domperidone (57.8 ± 6.6%) than two other groups, there was no significant difference between the groups.

DISCUSSION

The necessity of using inducing agents such as C.P.E., HCG and *GnRHa* for induction of spawning has been demonstrated in cyprinid fish such as common and chinese carps (Weil *et al*., 1986; Peter *et al*., 1988) as well as Indian major carps (Chaudhuri, 1976). No ovulated fish was observed in the group receiving *GnRHa* alone due to the strong dopamine inhibitory tone on pituitary GtH secretion in common carp. It has been previously demonstrated that combination of *GnRHa* with a dopamine receptor antagonist such as domperidone or metechlopramide is necessary for spawning induction in cyprinid fish such as common carp (Ghezel, 1993), loach *Paramisgurnus dabryanus* (Lin *et al*., 1987) and nase, *Chondrostoma nasus* (Szabo *et al*., 2002).

Ninty percent of fish ovulated in the group treated with *GnRHa*+domperidone in two injections, given 7h. apart, which was significantly higher than C.P.E. (7/16) or *GnRHa* +domperidone in one injection (3/12) treated groups. The higher spawning rate in fish receiving *GnRHa*+domperidone in two injections compared to C.P.E. treated fish, is probably due to the more effectiveness of synthetic hormones rather than the pituitary extracts and the exact dosage of this superactive compounds (Zohar, 1989). Using *GnRHa* + domperidone in one injection showed lowest spawning rate in common carp, this could be due to rapid clearance of *GnRHa* from blood circulation under the influence of cytosolic enzyme activities in the pituitary, kidney and liver, Zohar and Mylonas (2001) noted that the mean time activity of *GnRHa* is too short (approx. 23 min) and it is better to use multiple injections of aqueous solution or slow release delivery systems of *GnRHa* for successful induction of spawning in fish. However, they pointed out that a single injection of *GnRHa* + dopamine antagonist is usually sufficient for spawning induction in common as well as Chinese carps. Such variations are probably due due to the differences following factors, the stage of ovary maturation, genetic variability among broodstocks, the purity and kind of GnRH analogue and dopamine antagonist receptors .

The latency period is relatively higher in groups receiving *GnRHa* +domperidone compared to C.P.E. treated fish, although there are no significant differences. Probably C.P.E. acts on the gonads while *GnRHa* acts at a higher level of the reproductive axis. Another reason could be propylene glycol as a *GnRHa*+domperidone solvent cause lesser releasing of this compound in the blood circulation as compared to C.P.E. saline solution, which cause higher levels of latency period in *GnRHa* + domperidone treated fish (Zohar and Mylonas, 2001).

According to our results the working fecundity in spawned fish was approximately in the range of 50-150 thousands. Although it was higher in *GnRHa*+domperidone as compared to fish treated with C.P.E but there was no significant difference between *GnRHa*+domperidone injected fish. It appears that 3 mg/kg of C.P.E was capable of inducing significant changes in the ovary but it is not high enough to induce complete ovulation under given conditions. However it was reported that this dosage is effective for complete spawning process in female common carp (Zohar, 1989). In addition, previous studies did not show any difference between the percent of stripped eggs/kg b.w. in common carp (Ghezel, 1993), silver carp (Makhdomi, 1993) and grass carp (Ghanei Tehrani, 1993) treated either with *GnRHa*+metoclopramide or C.P.E. These differences are probably due to the variability in the pituitary glands potency and GtH concentration, which depend on the origin of C.P.E., harvesting time of the pituitary gland and storing conditions (Nandeesha *et al*., 1990).

Brzuska (1990) reported that the ovulation index in grass carp, *Ctenopharyngodon idella* and silver carp, *Hypophthalmichthys molitrix*, did not differ in females treated with adequate dose of *GnRHa*+pimozide or carp pituitary. The present study also showed that different doses of *GnRHa* in rainbow trout, *O. mykiss*, did not show any significant difference in the mean weight of stripped eggs×100/kg b.w. compared to untreated fish (Dorafshan *et al*., 2002).

Embryo viability didn't show any significant difference between groups, it is suggested that *GnRHa*+ domperidone didn't show any adverse effect on egg viability under experimental conditions in Ahwaz. Similar results were obtained on common carp

(Ghezel, 1993; Kulikovsky *et al*., 1996) and Indian major carps (Nandeesha *et al*., 1990). However, Szabo *et al*. (2002), pointed out that using *GnRHa* + domperidone in order to induce ovulation in nase (*C. nasus)* resulted in higher fertilization rate as compared to pituitary injected fish, they suggested that it maybe due to higher levels of *GnRHa* acting site at the reproductive axis compared to pituitary gland, inducing not only the release of endogenous GtH from the pituitary also other important indogenous hormone from pitutary.

In summary, this study demonstrated that the use of new Iranian made *GnRHa* coupled with domperidone is an effective and reliable procedure for induction of ovulation and spawning in *C. carpio* in two injections protocol in a total dose of 10 µg +5 mg/kg b.w. in 10-90% injection rate. This method has some advantages over hypophyseal treatments, such as higher working fecundity and the lower cost. However it is necessary to conduct more research on other warm water cultured fish to find out the best injection regime for each special treatments and comparing it with traditional methods.

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