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Study on the potency of Domperidone and Metoclopramide for spawning induction in Kutum (*Rutilus frisii kutum*)

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Abstract: The effectiveness of two kinds of dopamine antagonist (DA), metoclopramide (Met) and domperidone (Dom) combined with the gonadotropin releasing hormone analogue (D-Ala^6, Des Gly^{10} ethylamide) GnRHa was assayed on the ovulation success, latency period, ovulation index (OI) and fertilization success of kutum, *Rutilus frisii kutum* (Kamenskii, 1901). Broodfish were injected intra-peritoneally as follows: 2 mg/kg b.w. of carp pituitary extract (CPE) as a control, 5 μg + 2.5mg, 10μg + 5mg and 20μg + 10mg/kg b.w. of GnRHa + Met or Dom in a single injection.

Based on the ovulation and fertilization success, no significant differences between similar doses of Dom and Met when combined with similar doses of GnRHa were found ($P>0.05$). However, in some groups, the OI and latency period were greater when Dom was used as a DA instead of Met ($P<0.05$). In general, the results of this study showed that the potency of Dom was nearly same as Met when combined with GnRHa and used as a DA in kutum. It is strongly recommended to repeat the experiment under different conditions to find out definite conclusion.

Keywords: Kutum, *Rutilus frisii kutum*, Spawning, GnRHa, Metoclopramide, Domperidone.

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Introduction

Spawning induction techniques using hormonal intervention provide on-demand high quality and quantity of gametes that are necessary for constant development of aquaculture and restocking programs (Donaldson, 2003). Hypophysation technique using carp pituitary extract (CPE) is a traditional method applied for spawning induction in cyprinids with an unpredictable response (Drori *et al.*, 1994). An alternative technique is the Linpe method based on using gonadotropin releasing hormone analogue (GnRHa) combined with a dopamine antagonist, DA (Peter *et al.*, 1988), which is successfully applied in different cyprinids such as goldfish, *Carassius auratus* (Sokolowska *et al.*, 1984), bream, *Parabramis pekinensis* (Lin *et al.*, 1986), nase, *Chondrostoma nasus* (Szabo *et al.*, 2002), pearl mullet, *Chalcalburnus tarichi* (Arabaci and Sari, 2004) and common carp, *Cyprinus carpio* (Drori *et al.*, 1994; Yaron, 1995; Kulikovsky *et al.*, 1996; Dorafshan *et al.*, 2003; Arabaci *et al.*, 2004). The effective doses of GnRHa and DA varies in the range of 2–20μg/kg b.w. of GnRHa and 0–20mg/kg b.w. of DA based on species, maturation stage, sex, potency and kinds of hormone/drugs (Billard, 1990; Donaldson, 1999). Domperidone (Dom), metoclopramide (Met), pimozide, sulpride and haloperidone are the major DA inhibiting the dopaminergic effect in fish (Bromage *et al.*, 1993). Among them, Met has become a common candidate as a DA agent in fish because of some advantages like availability and solubility in aqueous vehicles; although its potency was inferior to that of Dom in some species like goldfish (Omeljaniuk *et al.*, 1987), it was successfully used in common carp (Drori *et al.*, 1994; Kulikovsky *et al.*, 1996), silver carp, *Hypophthalmichthys molitrix* (Kashani Sabet *et al.*, 2004) as well as kutum, *Rutilus frisii kutum* (Dorafshan & Paykan Heyrati, 2006). Another well-known DA is Dom which has the strongest inhibitory effect on dopamine action without the side effect caused by transportation through brain-blood barrier (Zohar, 1989; Donaldson, 2003) that used extensively for spawning induction of different fish species (Billard, 1990).

The objective of the current study was to assess the potency of Met and Dom for spawning induction in kutum.

Materials and Methods

Fish samples:

The experiment was conducted on kutum broodfish at Shahid Ansari Cyprinid Fish Hatchery, Rasht, Iran. Fish were captured from the Sefidrood inlets to the Caspian Sea during spawning migration in April-May 2004 (water temperature 8-12°C). Seventy ripe females weighing 400-1400g b.w. were selected, based on the softness of their abdomens. Prior to injections, fish were individually weighed and marked by placing visible tags on dorsal fin and randomly divided into seven groups of 10 individual each.

Hormones and Drugs:

A GnRH analogue (D-Ala^{6}, Des Gly^{10}) ethylamide and domperidone (Dom) were supplied by National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran. Metoclopramide, Met, (Hakim Co. Iran), was purchased from human drug store in solution form in distilled water (10mg/ml).

For fish receiving GnRHa + Dom, at first, Dom was dissolved (100mg/ml) in dimethyl sulfulxide, DMSO (Omeljaniuk *et al.,* 1987) then diluted (1:4) with propylene glycol (P.G.) which contained 67µg/ml GnRHa to achieve a concentration of 50µg GnRHa plus 25mg Dom at a final volume of 1 ml and the combination was used for injection. For groups treated with GnRHa + Met, the GnRHa was diluted (50µg/ml) in P.G. then, injected separately at the same time with Met treatment. 2mg/kg b.w. of CPE was used in 0.7% saline according to the local hatchery experiences.

Methods:

Groups of 10 fish were injected intraperitoneally (ip) with different preparations as follows: CPE, as control (2mg/kg b.w.), and GnRHa treatment combined with Met or Dom as shown in Table 1. All treatments were done in a single injection. After injection, fish were placed in an indoor concrete fish pond with running water of 11-12°C. The fish were checked for ovulation 48h after injection at every 8h interval up to 72h. Upon observation of ovulation, the eggs were stripped manually and fertilized with milt from at least two males to reduce the side effect of poor quality of semen on fertilization (Szabo *et al.,* 2002); about 70 ripe males without

hormonal treatment were used. Fertilized eggs (20-30g) from each female were incubated in jar incubators up to hatching.

Table 1: Dosage and hormonal preparations used for inducing spawning in kutum

Groups	Treatment*	Fish No.	Injection dosage /kg b.w.	Injection volume (ml/kg b.w.)
1	C.P.E.	10	2 mg	0.5
2	GnRHa + Met	10	5 µg + 2.5 mg	0.1 + 0.25
3	GnRHa + Dom	10	5 µg + 2.5 mg	0.1
4	GnRHa + Met	10	10 µg + 5 mg	0.2 + 0.5
5	GnRHa + Dom	10	10 µg + 5 mg	0.2
6	GnRHa + Met	10	20 µg + 10 mg	0.4 + 1
7	GnRHa + Dom	10	20 µg + 10 mg	0.4

*** C.P.E.: Carp pituitary extract, Met: Metoclopramide and Dom: Domperidone**

Assessment of spawning was carried out by determining the ovulation success (no of ovulated females/no of injected) and ovulation index (OI). In order to determine OI, females were sacrificed after ovulation and the ovaries or ovarian remnants were weighed and OI was calculated as weight of stripped egg mass/eight of stripped egg mass + remnant ovaries (Szabo *et al.*, 2002). Fertilization success was determined under a dissecting microscope 3 days after fertilization, when eggs were at the stage of gastrulation at least for 100 eggs for each female (Razavi Sayyad, 1984). The latency period defined as a time between injection and ovulation were calculated according to Drori *et al.* (1994).

Statistical analysis:

Ovulation success was analysed by Chi-square test (Szabo *et al.*, 2002). The differences in latency period, OI and fertilization success data were analysed by one way analysis of variance (ANOVA) at minimum significant of $P<0.05$. Results are presented as means ±SEM (Kulikovsky *et al.*, 1996).

Results

The results of the effects of hormonal treatment on ovulation success are summarized in Fig.1. All treatments could cause ovulation but the potencies were significantly different ($P<0.05$). Only two out of 10 fish were ovulated in groups

receiving GnRHa + Met or Dom at the lowest dose (groups 2 & 3), which were significantly lower than CPE treated control group ($P<0.05$). The median dose of compounds (groups 4 & 5) showed similar results to control, while the maximum doses of GnRHa + DA increased ovulation success up to 90 and 100% (groups 6 & 7) that were significantly higher ($P<0.05$) than all other groups including control (60 %).

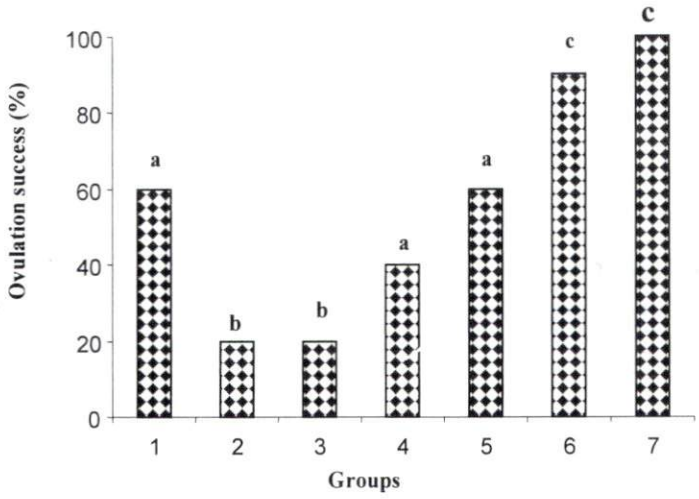

Figure 1: Ovulation success (%) in kutum following hormonal treatment, the best results were obtained when GnRHa and Dom or Met used at a highest dose (group 6 & 7). The groups designated by the same letters are not significantly different ($P>0.05$).

The latency periods varied in the range of 48-72h post injection (Fig. 2). The shortest latency period (48±1h) was observed in the group 3. By increasing the GnRHa and DA doses, the latency periods became longer and the longest was achieved in fish receiving GnRHa 10µg + Dom 5mg (group 5). The mean latency period in CPE treated fish was 52±6h and it was similar to periods in groups 4 and 6 ($P>0.05$).

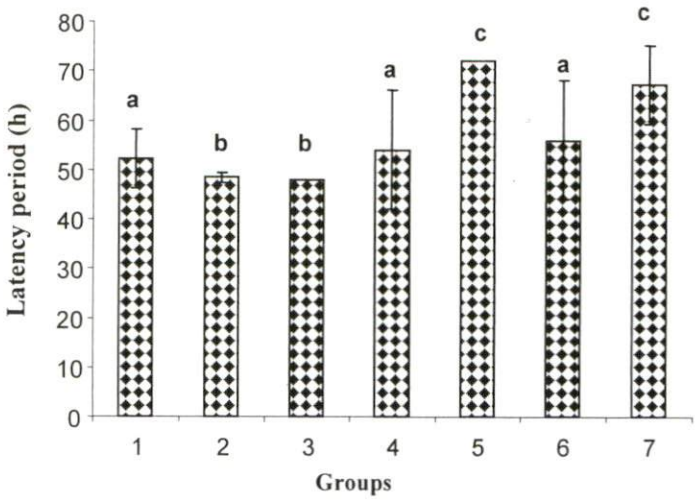

Figure 2: Latency period (h) in kutum following hormonal treatments. The longest and shortest latency periods were achieved in groups 7 and 2 respectively. The groups designated by the same letters are not significantly different (P>0.05).

The OI was in the range of 60-83%, showing great differences among groups (Fig. 3). The lowest OI was in the group 2 & 3, which received GnRHa + Met or Dom at the lowest doses. The best OI (83±4%) was achieved in the group 7 (GnRHa + Dom in the highest dose), which was similar to the OI values in the groups 5 and CPE treated control (P>0.05).

Fertilization success in treated fish was in the range of 67-76%, the normal range of the hatchery practice of kutum as well (Fig. 4). Although the lowest and highest fertilization rates were observed in groups 1 & 5, there were no significant differences among groups (P>0.05).

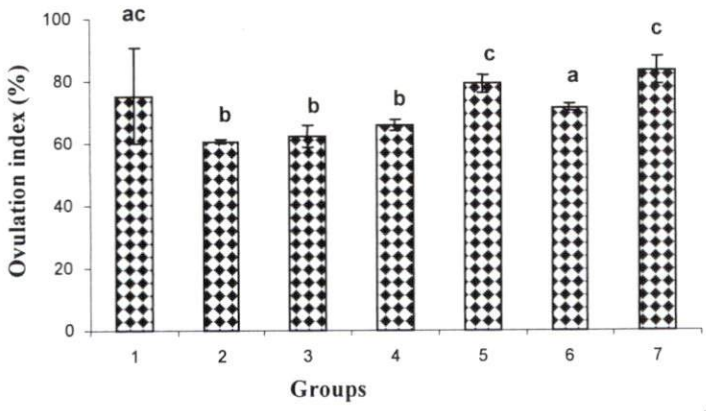

Figure 3: Ovulation index (%) in kutum following hormonal treatments. The lowest and highest ovulation indexes were achieved in groups 2 and 7 respectively. The groups designated by the same letters are not significantly different (P>0.05).

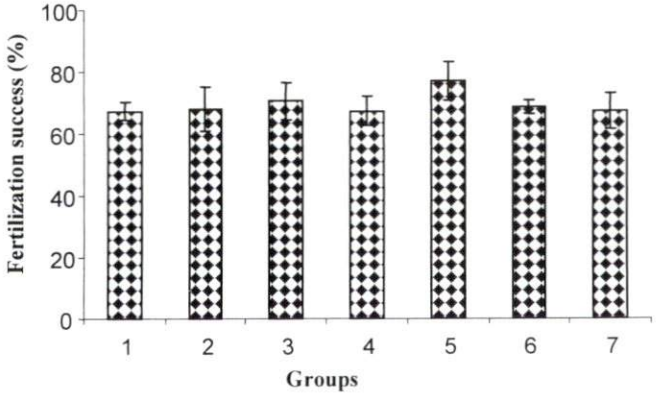

Figure 4: Fertilization success (%) in kutum following hormonal treatments. There are no significant differences among groups (P>0.05).

Discussion

All treatments could induce ovulation in kutum, showing that the fish were in the preovulatory stage at a time of hormonal injection. The best ovulation successes were observed in groups receiving GnRHa and Met or Dom at the highest doses (groups 6 & 7). Decreasing the compound dosage resulted in a significant reduction of ovulation, although no significant differences found between similar doses of Met or Dom combined with parallel doses of GnRHa (Fig. 1). So, it can be concluded that Met and Dom have a similar effect on ovulation success of kutum when their similar doses are combined with similar doses of GnRHa. This finding clearly differs from those described by Omeljaniuk *et al.* (1987) on goldfish, Drori *et al.* (1994) and Kulikovsky *et al.* (1996) on common carp and Kashani Sabet *et al.* (2004) on silver carp. They stated that Met dosage must be 3-4 times more than doses of Dom at a same dose of GnRHa to stimulate ovulation similarly. This may be due to the difference in physiological conditions and/or species specificity of dopaminergic effect on GtH secretion. The dopaminergic effect completely differs according to fish species and physiological conditions (Zohar & Mylonas, 2001). Donaldson (1999) suggested that the suitable doses of DA in various fish species lie in the range of zero in salmonids, up to 20mg/kg b.w. in some cyprinids like common carp depending on kind of DA, maturation stage, sex and GnRH dosage. The other probable variables were temperature, fish size, age or other environmental conditions.

Lower temperature (7-15°C) in spawning season of kutum compared to other well-known cyprinids may cause longer latency periods of this species (Fig. 2). It was shown that higher temperature after hormonal treatment reduced latency periods in fish (Billard, 1990; Drori *et al.*, 1994). The latency periods tended to be longer in hormonal treated groups (except groups 2 & 3) in comparison with CPE treated fish (Fig. 2). It was also observed in our previous study on common carp (Dorafshan *et al.*, 2003) and kutum (Dorafshan & Paykan Heyrati, 2006). This

phenomenon can be explained by higher level of acting site of GnRHa in comparison with gonadotropin hormone (GtH), the main hormone in CPE causing ovulation, in the reproductive axis. Using propylene glycol as Dom solvent that causes slower releasing of the compounds compared to aqueous solution of Met can probably explain general longer latency periods in Dom instead of Met treated fish at a same dose of GnRHa (Sato *et al.*, 1995).

The OI is a very suitable index in calculating the efficiency of hormonal treatment for ovulation induction in fish (Szabo *et al.*, 2002). The lowest OI was found in groups receiving the lowest doses of GnRHa+Met or Dom (Fig. 3). Increase in the hormonal dosages resulted in increased OI; the maximum OI was observed in groups treated with the highest dosage of GnRHa and Dom (group 7). The groups treated with the lower doses of GnRHa+Met or Dom generally showed lower OI (Fig. 3), probably due to failure in stimulating an adequate increase in circulating GtH levels, because inadequate dose or activity of DA or GnRHa. Similar results have also been found in pike, *Esox lucius* (Billard & Marcel, 1980) as well as nase (Szabo *et al.*, 2002), which have reportedly been due to inadequate dose of CPE.

Fertilization success did not show any significant difference among groups (Fig. 4). It is suggested that Dom or Met do not have any adverse effect on egg viability (Zohar, 1989). Similar results have been reported on common carp (Drori *et al.*, 1994; Kulikuvsky *et al.*, 1996; Dorafshan *et al.*, 2003) and pearl mullet (Arabaci & Sari, 2004). It is completely accepted that using GnRHa and/or DA in a wrong time, not at preovulation stage, or using very high doses of them can cause great adverse effect on egg quality (Bromage & Cumaranatunga, 1988; Mylonas *et al.*, 1992).

In conclusion, this study demonstrated that except for some differences in the OI, Dom and Met have similar efficiency when combined with GnRHa for spawning induction in female kutum. However, it is strongly recommended to

repeat the experiment with the larger numbers of kutum broodfish to find out the differences more clearly.

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