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Ovulation and spawning induction in *Rutilus frisii kutum*: Advances in application of catecholaminergic pharmaceutics at different doses as compared with ovaprim a potent ovulating agent

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Abstract
The involvement of adrenergic and dopaminergic receptor subunits are recognized in regulating fish reproduction. The potential for neuroendocrine novel pharmaceutics to induce final maturation and ovulation has not been well studied. The aim of the present study was to investigate the effects of new catecholamine pharmaceutics at different doses, including clozapine and olanzapine (the third generation of D_4 and D_2 dopamine receptor antagonists, respectively), which could be more effective than the first and second generations, salbutamol (β_2-adrenergic receptor agonist) and metoprolol (β_1- adrenergic receptor antagonist) as compared with ovaprim (D-Ala^6,Pro^9-Net)-sGnRH+Domperidone, on final oocyte maturation (FOM), ovulation and spawning in *Rutilus frisii kutum*. In the present investigation, 126 gravid females were injected once with ovaprim, 0.5mlkg^{-1} BW, salbutamol sulphate (SLB) 2, 4 or 8 mg kg^{-1} BW; metoprolol tartrate (MTP) 5, 10 or 20 mg kg^{-1} BW; olanzapine (OLZ) 2, 5 or 10 mg kg^{-1} BW; clozapine (CZ) 2, 8 or 12 mg kg^{-1} BW (N=9), been divided into 14 groups. Intact and ovaprim were negative and positive Control groups respectively. The results showed that all fish dosed with 0.5 ml kg^{-1} of ovaprim ovulated within 13 hours after injection,while no fish ovulated in negative control group (Intact). The parameters include ovulation index, fertilization success, relative fecundity and the number of eggs which were the highest mean values belonged to ovaprim treatment. Ovulation success (100%) was obtained in OLZ 5 mg kg^{-1}, CZ 12 mg kg^{-1}, MTP 5 mg kg^{-1} and SLB 4 mg kg^{-1} treatments. Therefore, it could be concluded that SLB 4 mg appears to employ β_2-adrenoceptor mechanisms in mediating the considered effects. In contrast, MTP at high doses (10 mg and 20 mg) significantly inhibited the stimulatory effect of adrenergic system on FOM and ovulation. The stimulation by the blocker (MTP) was higher in the low dose, 5 mg kg^{-1}. The successful spawning using CZ 12 mg and OLZ 5 mg are important steps in the expansion of the culture of Caspian kutum.

Keywords: New pharmaceutics; Ovaprim; Ovulation; Optimum dose; Caspian kutum

1. Introduction

The hypothalamic–pituitary–gonadal (HPG) axis is a principal neuroendocrine pathway regulating reproductive processes in teleosts as in other vertebrates. Hypothalamus region stimulates gonadotropin-releasing hormone (GnRH) synthesis and release, and GnRH controls the secretion of gonadotropin hormone (GtH) from the pituitary (Zohar et al. 2009). Two types of gonadotropins, follicle-stimulating hormone (FSH, GtH-I) and luteinizing hormone (LH, GtH-II) regulate gonadal functions, such as gametogenesis and steroidogenesis (Swanson et al., 2003). Both gonadotropins stimulate ovarian steroid hormones synthesized by the follicular cells surrounding the oocyte and appear to be present throughout the reproductive cycle. 17β-estradiol (E_2) and 17α,20β-dihydroxy-4-pregnen-3-one (17,20β-P) are the major sexual steroids controlling ovarian development and maturation (Sen et al., 2002).

Catecholamine messengers play important roles in the regulation of the physiological reproductive cycle in fish. In many teleosts *i.e.* cyprinids, the gonadotrophs are directly innervated by both peptidergic- and aminergic- neurons (Perez, 2005). In fact, monoaminergic systems may be involved in HPG axis and the presence of β-adrenoceptors as the targets of circulating catecholamine has been reported in some studied fish (Fabbri et al., 2001). The catecholamines show considerable variations in their functions as reported in several species (Crim et al., 1989; Manickam & Joy, 1990; Senthilkumaran & Joy, 1993). Investigations show clearly a remarkable increase in the contents and activity of noradrenaline (NA) and specially adrenaline (A), during the progress of ovarian development and final maturation, while a corresponding decrease in dopamine (DA) activity (Lee et al., 2000) occurs. In fact, monoamines like NA and serotonin (5-HT), in contrast to DA, stimulate GtH-II secretion.

Adrenoceptors mediate the essential neurohormones, NA and A which play main roles in the regulation of various physiological functions and involved in ovarian development, such as steroidogenesis, folliculogenesis and ovulation in vertebrates (Miszkiel & Kotwica, 2001; Kotwica et al., 2002). The β-adrenergic receptors (β-ARs) are members of the specific membrane G-protein-coupled receptors (Owen et al., 2007). There are three known β-ARs: β_1, β_2 and β_3 (Kaumann et al., 2001).

DA is a principal catecholaminergic neurotransmitter which is involved in many functions of the nervous system. DA is an inhibitory neurohormone in fish reproduction, which acts to inhibit GnRH release from GnRH neurons and GtH release from pituitary cells (Yaron et al., 2003). In fact, the DA-ergic inhibitory effect on GtH-II release has been recognized in many species (Vidal et al., 2004; Dufour *et al*., 2005; Patiño & Sullivan, 2002). DA acts directly onto the gonadotrophs through D_2 receptors (Vacher et al., 2000). DA receptors have been classified into two groups: D_1-like and D_2-like. D_2-like DA receptors include D_2, D_3 and D_4-DA receptors (Neve, 2009). D_2-like antagonists have an important role in the reproductive cycle in teleosts. Dopaminergic pharmaceutical compounds categorize in the first, second and third generations in which the third generation of DA antagonists are more effective and sensitive in comparison with the first and second ones (Maguire, 2002). Clozapine and Olanzapine are related to the third one (Maguire & Nemec, 2001).

In some species, hormonal manipulations are the only technique of controlling reproduction reliability. Over the years, a diversity of hormonal approaches has been successfully used (Zohar & Mylonas, 2001; Mylonas et al., 2010; Mikolajczyk et al., 2004). Hormonal inductions have main applications in commercial aquaculture, even for fish which do

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undergo final maturation and ovulation in captivity, so it could advance maturation and ovulation during few weeks. Today, various synthetics, highly potent agonists and antagonists of catecholaminergic systems are available. These procedures have contributed significantly to the development of more reliabile fish reproductive procedures (Koohilai et al., 2017).

Caspian kutum, *Rutilus frisii kutum*, is a migratory anadromous species and one of the most valuable commercial and endemic cyprinid fish in the Caspian Sea. This kind of fish spawns in rivers inlet to the Caspian Sea from March to May (Sharyati, 1993). The aim of this study was to investigate ovulation and spawning induction by pharmaceutics administration belong to β_1- and β_2-adrenoceptors and D_2-like antagonists at different doses, under artificial conditions through measuring reproductive parameters. Considering the importance of the catecholaminergic system in fish reproduction, achieving optimum doses of catecholamine agonists and antagonists, which are related to the new generation, can be used in the aquaculture industry. This could be done through treatments in which, they are combined with GnRH analogues and can be presented in the form of synthetic kits for aquaculture. Therefore, achieving effective doses in this study will help us to reach our future goals to enhance rebuilding the kutum reserves in the Caspian Sea.

2. Material and Methods

2.1. Animal collection and acclimatization

Gravid females *Rutilus frisii kutum* (*n*=126) were captured from the Shirud River inlets to the Caspian Sea in April 2014, then they were transported to the laboratory under artificial conditions, *i.e.* constant oxygenation, temperature 12.4 ± 0.5 °C and artificial photothermal conditions including ambient photoperiod (12-13 hours light). Light intensity value throughout the entire experiment was around 300 Lux. They were maintained in tanks (diameter 2.33 m and depth 45 cm, volume 1.92 m^3), weighed (1062.7 ± 51.90 g) and assigned based on the separate treatment groups.

2.2. Chemicals

Ovaprim *(D-Ala^6, Pro^9- Net) -sGnRH* + *Domperidone* (Syndel Laboratories, India) was purchased from Virbac Animal Health India company. Salbutamol sulphate (*4-[2-(tert-butylamino)-1-hydroxyethyl]-2-(hydroxymethyl)phenol*) was purchased from Neuland Laboratories Limited India company. Metoprolol tartrate (*1-[4-(2-Methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]-2-propanol hemitartrate*) was purchased from Sunpharma India company. Clozapine (*8-Chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo[b,e][1,4]diazepin*) was purchased from Cambrex Profarmaco Milano S.r.l company and Olanzapine (*LY170053,2-Methyl-4-(4-methyl-1-piperazinyl)-10H-thieno[2,3-b][1,5]benzodiazepine*) was purchased from Lee Pharma Limited India company.

Ovaprim is a commercial spawning inducer for the artificial breeding of fish. GnRH-III is denoted for sGnRH. Salbutamol is a short-acting β_2-adrenergic agonist. Ovary innervated by adrenergic terminal nerves and in all parts of the ovary, the β_2-adrenergic fibers were most numerous. Metoprolol is a β_1-selective blocker. Many of the B_1-AR blockers will also block B_2-receptors if present in higher concentrations. Clozapine as an atypical neuroleptic drug binds to several types of central nervous system receptors. It also displays a strong affinity to several dopaminergic receptors (higher affinity for the D_4- than for D_2- and D_3-DA receptors) and displays a unique pharmacological profile. Olanzapine is a synthetic derivative of thienobenzodiazepine, binding not only D_2-DA receptors, but also serotonergic 5-HT_2-receptors as well as the adrenergic α_1- and the histamine H_1-receptors.

2.3. Experiments

The acclimatized females *Rutilus frisii kutum* were randomly divided into 14 groups, Group 1: Intact (negative Control), Group 2: Ovaprim (positive Control), Group 3: SLB 2 mg kg^{-1} BW, Group 4: SLB 4 mg kg^{-1} BW, Group 5: SLB 8 mg kg^{-1} BW, Group 6: MTP 5 mg kg^{-1} BW, Group 7: MTP 10 mg kg^{-1} BW, Group 8: MTP 20 mg kg^{-1} BW, Group 9: CZ 2 mg kg^{-1} BW, Group 10: CZ 8 mg kg^{-1} BW, Group 11: CZ 12 mg kg^{-1} BW, Group 12: OLZ 2 mg kg^{-1} BW, Group 13: OLZ 5 mg kg^{-1} BW, Group 14: OLZ 10 mg kg^{-1} BW. Intramuscular injection (IM) was performed by penetration of the dorsal muscles at the level of dorsal fin (Schreck, 1973).

After injection, the fish were placed in separated tanks and checked for ovulation by hand-stripping, 10 hours after injection and this process was repeated every one hour. When ovulation occurred, the ovulated eggs flow out easily in a thick jet from the genital vent and were usually collected into a dry bowl and rinsed with fresh water. Afterwards, the number of eggs was measured and fertilized in vitro with the milt collected from at least two mature males. Spermiation in the males was verified by applying gentle pressure on the abdomen to extrude the milt onto the eggs. The sperm was added to the stripped eggs and fresh milt was spread over the eggs. The eggs were fertilized by adding an equal volume of freshwater. The water and egg mass have been then mixed by gently shaking the bowl. One thousand four hundred eggs were fertilized with 10 cc semen containing 2 million sperm per cubic centimeter. Water was added to cover the eggs so that the eggs swelled up. After several minutes, the fertilized eggs of the individual broodstock females were rinsed with fresh water for 45 min to eliminate egg stickiness before transferred to the incubators.

Appraisement of ovulation was carried out by ovulation success, determination (number of ovulated females/number of injected ones) ×100% and ovulation index (*OI*) weight of stripped egg mass/(weight of stripped egg mass + remnant ovaries) ×100% (Szabó et al., 2002). Fertilization success was defined under a dissecting microscope, 24 hours after fertilization when eggs were at the stage of gastrulation (Razavi Sayyad, 1984). The percentage of egg weight to body weight was determined as follows: (Weight of stripped eggs mass in each treatment/total weight of females) ×100%. Total ovulated eggs in Caspian kutum were placed in the abdominal cavity so they were accessible and released spontaneously. Accordingly, relative fecundity was calculated by (total number of eggs in each group/total weight of females) at the post-spawning period for the eggs collected in each treatment (Prat et al., 2001).

2.4. Statistics

Data were expressed as mean ± standard error of the mean (S.E.M). Bodyweight, total length, ovulation index, ovulation success, the number of eggs, fertilization success, relative fecundity and the percentage of an egg’s weight to body weight were calculated. The normality of the variance was tested by the Kolmogorov–Smirnov. Statistical differences between experimental groups were examined using a one-way analysis of variance (*ANOVA*, $P<0.05$) followed by *Tukey’s HSD* post-test.

3. Results

3.1. Effects of ovaprim administration on the induction of ovulation and spawning

All the fish ovulated (100%) in the positive Control group (ovaprim), and the response (latency period) was evident at 13 hours of post-injection,while no fish ovulated in negative Control group (Intact). Ovaprim treatment resulted in significant elevations in ovulation index, fertilization success, relative fecundity, the number of eggs and egg weight to body weight (Table 1).

3.2. The appraisement of different doses of salbutamol (β2-adrenergic agonist) on reproductive parameters

Treatment with SLB 4 significantly differed (*P*<0.01) the ovulation and spawning (100%) at 17 hours after injection. The treatment of females with ovaprim and SLB 8 mg enhanced ovulation index (*P*<0.05). SLB 8 mg and ovaprim treatments significantly increased (*P*<0.01) relative fecundity to compare with other doses. There was no significant difference in the mean fertilization success among groups (*P*>0.05, Table 1).

3.3. The evaluation of different doses of metoprolol (β1-adrenergic antagonist) on reproductive parameters

Ovulation success was significantly increased (100%) after treatment with MTP 5 mg (*P*<0.01) at 11 hours after injection, while spawning was reduced in high doses of metoprolol (MTP 10 and MTP 20 mg; *P*>0.05). For ovulation index values, the influences of hormonal treatments turned out to be significant after ovaprim and MTP 5 mg application in comparison with other treatments (*P*<0.05). Treatment with MTP 10 mg surprisingly reduced most of the reproductive parameters (*P*<0.001). For the success of ovulation, ovulation index, relative fecundity, the number of eggs and percentage of egg weight to body weight parameters exhibited a considerable range, inclusive 33%, 60%, 8.9 (eggs g^1), 12000 and 3.73% respectively. There were no significant differences in fertilization success and the latency period between all groups (*P*>0.05, Table 2).

Table 1: The effect of salbutamol at different doses on ovulation success (%), ovulation index (%), fertilization success (%), relative fecundity (eggs g^{-1}), the number of eggs, egg weight to body weight (%) and latency period (h) in Caspian kutum, *Rutilus frisii kutum*.

Parameter	**Intact**	**Ovaprim**	**SLB 2**	**SLB 4**	**SLB 8**
Body Weight (g)	831.1±84.08	1341.7±220.4	1283.3±38.54	820±87.17	830±17.21
Total Length (cm)	44.2±1.5	50.4±2.6	49.3±3.9	43.3±1.2	44±1.5
Ovulation Success (%)	______	100^{c*}	67^{b}	100^{c*}	67^{b}
Ovulation Index (%)	______	$84.3±0.3^{b}$	$72±7^{a}$	$70.3±2.4^{a}$	$80±5^{b}$
Fertilization Success (%)	______	93.1±0.7	90±2	89.6±0.8	90.5±1.5
Relative Fecundity (eggs g^{-1})	______	$57.05±13.2^{c*}$	$26.89±15.8^{a}$	$25.44±7.6^{a}$	$51.35±12.6^{c*}$
The number of eggs	______	$67842±25623^{c*}$	$24860±14060^{a}$	$34167±13090^{a}$	$47860±14200^{b}$
Egg Weight to Body Weight (%)	______	$17.9±2.3^{c*}$	$9.73±5.5^{a}$	$8.35±4.2^{a}$	$8.88±6.1^{a}$
Latency Period (h)	______	13^{b}	12^{b}	17.33^{a}	18^{a}

[b]Mean (±SE) values with a different superscript are significantly different (P<0.05).

[c*]Mean (±SE) values with a different superscript are significantly different (P<0.01).

Table 2: The effect of metoprolol at different doses on ovulation success (%), ovulation index (%), fertilization success (%), relative fecundity (eggs g^{-1}), the number of eggs, egg weight to body weight (%) and latency period (h) in Caspian kutum, *Rutilus frisii kutum*.

Parameter	**Intact**	**Ovaprim**	**MTP 5**	**MTP 10**	**MTP 20**
Body Weight (g)	831.1±84.08	1341.7±220.4	1283.3±38.54	820±87.17	830±17.21
Total Length (cm)	44.2±1.5	50.4±2.6	49.3±3.9	43.3±1.2	44±1.5
Ovulation Success (%)	______	100 ^{c*}	100^{c*}	33^{a}	33^{a}
Ovulation Index (%)	______	84.3±0.3^{b}	81.3±4.9^{b}	60^{a}	67.3±1.4^{a}
Fertilization Success (%)	______	93.1±0.7	88.6±1.8	90	88.3±1.8
Relative Fecundity (eggs g^{-1})	______	57.05±13.2 ^{b}	52.41±16.6 ^{b}	8.9 ^{c**}	32.39±4.4 ^{a}
The number of eggs	______	67842±25623 ^{c*}	50687±87180 ^{b}	12000 ^{c**}	34860±34017 ^{a}
Egg Weight to Body Weight (%)	______	17.9±2.3 ^{b}	17.81±1.7 ^{b}	3.73 ^{c**}	12.11±1.9 ^{a}
Latency Period (h)	______	13	11.33	15	12.33

^{b}Mean (±SE) values with a different superscript are significantly different (P<0.05).

^{c*}Mean (±SE) values with a different superscript are significantly different (P<0.01).

^{c**}Mean (±SE) values with a different superscript are significantly different (P<0.001).

3.4. The assessment of different doses of clozapine (D2-like antagonist) on reproductive parameters

Ovulation reached 100% at 15 hours post-injection (latency period) in the fish receiving the highest dose of CZ (12 mg). Regardless of 100% ovulation obtained in CZ 8 mg treatment, latency period (18 hours) suggests a prolonged response time to compare with CZ 12 mg group in this treatment. As a result, a significant difference (*P*<0.05) was observed in the parameter mentioned above (latency period) between both groups. Regarding the ovulation index, no statistical differences were found between the experimental groups (*P*>0.05), which was the highest mean value reached in CZ 12 mg treatment. Analyses of the number of eggs revealed significant differences in ovaprim group (*P*<0.01) as compared with other treatments. Statistically difference (*P*<0.01) detected in CZ 12 mg treatment for relative fecundity among the groups. No significant difference was found between all groups for successful fertilization parameter (*P*>0.05, Table 3).

3.5. The evaluation of different doses of olanzapine (D2-like antagonist) on reproductive parameters

The highest mean ovulation success value (100%), was recorded for OLZ 5 mg treatment with a latency period at 15 hours of post-administration, and significant differences (*P*<0.01) were noted in OLZ 5 mg and ovaprim treatments in comparison with other groups. Accordingly, OLZ 5 mg was known as the optimal dose. No significant differences were detected among all treatments for mean ovulation index (*P*>0.05). However, significant differences were observed in ovaprim (*P*<0.01) and OLZ 5 mg (*P*<0.05) in comparison with other treatments for the mean number of eggs. Additionally, a significant difference in relative fecundity was found between the ovaprim and other treatments (*P*<0.01). Fertilization success did not differ significantly (*P*>0.05) between groups. Treating with ovaprim significantly improved (*P*<0.05) the percentage of egg weight to body weight (Table 4).

Table 3: The effect of clozapine at different doses on ovulation success (%), ovulation index (%), fertilization success (%), relative fecundity (eggs g^{-1}), the number of eggs, egg weight to body weight (%) and latency period (h) in Caspian kutum, *Rutilus frisii kutum*.

Parameter	**Intact**	**Ovaprim**	**CZ 2**	**CZ 8**	**CZ 12**
Body Weight (g)	831.1±84.08	1341.7±220.4	1283.3±38.54	820±87.17	830±17.21
Total Length (cm)	44.2±1.5	50.4±2.6	49.3±3.9	43.3±1.2	44±1.5
Ovulation Success (%)	______	100 ^{c*}	67 ^{b}	100 ^{c*}	100 ^{c*}
Ovulation Index (%)	______	84.3±0.3	82.5±7.5	79±5.5	87±4.04
Fertilization Success (%)	______	93.1±0.7	87.5±2.5	87.3±1.4	89.6±0.8
Relative Fecundity (eggs g^{-1})	______	57.05±13.2 ^{a}	64.12±1.5 ^{b}	51.41±9.6 ^{a}	74.08±15.98 ^{c*}
The number of eggs	______	67842±25623 ^{c*}	59710±30190 ^{b}	43633±12412 ^{a}	56427±7943 ^{b}
Egg Weight to Body Weight (%)	______	17.9±2.3 ^{a}	22.17±7.7 ^{b}	17±2.4 ^{a}	26.5±7.2 ^{b}
Latency Period (h)	______	13 ^{b}	17 ^{a}	18.3 ^{a}	15 ^{b}

^{b}Mean (±SE) values with a different superscript are significantly different (P<0.05).

^{c*}Mean (±SE) values with a different superscript are significantly different (P<0.01).

Table 4: The effect of olanzapine at different doses on ovulation success (%), ovulation index (%), fertilization success (%), relative fecundity (eggs g^{-1}), the number of eggs, egg weight to body weight (%) and latency period (h) in Caspian kutum, *Rutilus frisii kutum*.

Parameter	**Intact**	**Ovaprim**	**OLZ 2**	**OLZ 5**	**OLZ 10**
Body Weight (g)	831.1±84.08	1341.7±220.4	1300±9.83	1030.7.6±156.5	900±10.96
Total Length (cm)	44.2±1.5	50.4±2.6	45±4.5	46.3±1.7	44±1.5
Ovulation Success (%)	______	100 ^{c*}	67 ^{b}	100 ^{c*}	33 ^{a}
Ovulation Index (%)	______	84.3±0.3	80	74±4	76
Fertilization Success (%)	______	93.1±0.7	91	87.5±2.5	92
Relative Fecundity (eggs g^{-1})	______	57.05±13.2 ^{c*}	46.13±1.5 ^{a}	43.34±9.4 ^{a}	31.89 ^{b}
The number of eggs	______	67842±25623 ^{c*}	37430±16910 ^{a}	46187±13633 ^{b}	35400 ^{a}
Egg Weight to Body Weight (%)	______	17.9±2.3 ^{b}	14.76±0.8 ^{a}	14.7±2.4 ^{a}	13.51 ^{a}
Latency Period (h)	______	13	17	15	15

^{b}Mean (±SE) values with a different superscript are significantly different (P<0.05).

^{c*}Mean (±SE) values with a different superscript are significantly different (P<0.01).

4. Conclusions

In the present study, results are consistent with the notion that D_2-like antagonists increased the number of females completing FOM and ovulation in captivity. In fact, this study is the first to apply novel pharmaceutics as the third generation in the dopaminergic system, which can be more efficient than the first and second ones. The ovulation-inducing potential of ovaprim is similar to already reported data in this species (Falahatkar et al., 2013; Heyrati et al., 2007). The most significant observation of the study is that the β-adrenoceptor blocker (MTP) induced FOM and ovulation using just a low dose. Ovulation was sensitive to MTP administration, so that significant decreases in FOM, ovulation and spawning, was observable at concentrations of high doses.

The ovaprim treatment exhibited 100% ovulation, suggesting that ovaprim accelerated FOM, ovulation and spawning (Chaube et al., 2014; Sharaf, 2012; Żarski et al., 2009; Falahatkar et al., 2013; Heyrati et al., 2007). Since, ovaprim contains a sGnRH analogue and D_2 receptor antagonist (domperidone), was effective in several fish species and stimulates the synthesis and release of the neuropeptide by the nucleus preopticus (Singh & Joy, 2008; Podhorec & Kouril, 2009).

An interesting observation of the present study was the proving effect of β-adrenoceptors in the ovary, suggesting the incidence of an alternate catecholamine pathway. The presence of adrenergic receptors (α and β) has been reported in fish (Ng et al., 1997). Moreover, in other studies, β-adrenoceptor has been shown to play an important role in the reproductive cycle (Kaumann et al., 2001), also NA activity was retained high level in the pre-spawning and spawning phases in fish (Singh et al., 2010). In the present study, salbutamol as a β_2-adrenergic agonist (Perez, 2005), had a stimulatory effect on FOM and ovulation, an average of 17 hours from injection to ovulation (100%) was observed by SLB 4 mg administration in the Caspian kutum. However, Singh et al. (2010) have indicated, the attendance of catecholamines: DA, noradrenaline and adrenaline in the catfish ovary. So, DA inhibits brain and plasma vasotocin levels, while noradrenaline stimulates it (Singh et al., 2013). The drop in DA activity at 17 hours after SLB 4 mg administration coincided with maturational activity, ovulation and spawning which increased the activity of adrenaline and β-adrenoceptors so that the decreasing DA tone coincided with the increasing adrenergic tone (Senthilkumaran & Joy, 1994). Also, the study by Jalabert (1976) indicated that adrenaline stimulated FOM and ovulation in female trout. Our study was more commonly reported that salbutamol at all doses had relatively little effect on β_2-adrenoceptor. The relatively lower values of SLB in comparison with ovaprim were detected in the current trial. Although, our previous study showed that SLB 4 mg in combination with GnRHa and D_2-like antagonist, had the most effective response to ovulation success (Koohilai et al., 2017).

The present information on catecholamine kinetics, like the adrenergic system in the kutum ovary will prepare a basis for experimental studies on the neural control of the ovary, as in other fish species and vertebrates. The data showed that β_1-adrenoceptor antagonist (MTP) activity responded differently during FOM and ovulation. The β_1-adrenoceptor activity was maintained high up after injection of MTP 5 mg, which characterized the completion of meiotic maturation and ovulation, although these parameters decreased extremely after injection of MTP 10 mg and 20 mg. The involvement of catecholaminergic receptors has been inquired in several studies. In these studies, β-adrenoceptor has been shown to play the main role, so that propranolol, the β-adrenoceptor antagonist inhibited germinal vesicle breakdown (GVBD) and ovulation in catfish (Senthilkumaran & Joy, 2001). β-Blockers alike metoprolol and propranolol acted within the adrenergic system which involved in energy mobilization, reproduction and etc. (Dzialowski et al., 2006; Owen et al., 2007). The mammalian adrenergic system may structurally and functionally be similar to the fish adrenergic system (Massarsky et al., 2011). At 11 hours after injection of MTP 5 mg, with an increase in β-adrenoceptors activity, ovulation success reached 100%. The number of eggs as well as other parameters including ovulation success, ovulation index, relative fecundity, latency period and egg weight to body weight, had the highest value of MTP 5 mg treatment. Similar results have been found by Dzialowski et al. (2006) suggesting that reproduction was sensitive to propranolol and metoprolol, hence reproductive efficiency and fecundity were extremely decreased.

Hormonal stimulation was necessary to induce FOM in many cyprinids (Brzuska, 2006; (Kucharczyk et al., 2008; Falahatkar et al., 2013; Koohilai et al., 2017; Heyrati et al., 2007). In cyprinid fish, such as kutum, catecholamine drugs tended to increase slightly and immediately before controlled spawning. It tended to confirm the important role of D_2-like antagonists in FOM. In some species, DA provides a negative control of pituitary GTH release (Peter & Yu, 1997). In fact, the application of a proper dose of CZ (12 mg) leads to 100% spawning success (). The latency of response to artificial propagation by CZ 12 mg in Caspian kutum was noted in appropriate time (15 hours). Other researchers have reported that the presence of a direct dopaminergic inhibitor (α-methyl-para-tyrosine or carbidopa) influences on gonadotrophs in females at FOM, indicating that DA has gonadotropin release-inhibitory factor (GRIF) activity (Sébert et al., 2008; Levavi-Sivan et al., 2010; Heyrati et al., 2007). Furthermore, Singh et al. (2010) reported that in the ovary, DA activity attained the perigee in the spawning phase and was preceded by high activity in the pre-spawning and post-spawning phases. In the present study, CZ 12 mg has increased FOM and ovulation in females. In addition, clozapine is rapidly distributed. It crosses the blood-brain barrier and is distributed in the circulatory system. The ovulation index and relative fecundity in case of kutum, were only high in CZ 12 mg treatment. In fact, approximately 87% of oocytes were ovulated after CZ 12 mg injection. A similar result was noted in goldfish by SKF-83566 (DA antagonist) administration, indicating that GnRH release was significantly inhibited from preoptic anterior hypothalamus (Yu & Peter, 1992). Results suggested that (i) dopamine inhibits FOM, ovulation and spawning and (ii) dopamine D_2-receptors in the pituitary are involved in this inhibition.

Olanzapine greatly inhibits the DA-D_2 receptor. It can bind not only dopamine D_2-receptors, but also, histamine H_1-receptors as well as α_1- and α_2-adrenoceptors. This blocker has been introduced the third generation of DA antagonists (Neve, 2009). In Caspian kutum, only treatment with OLZ 5 mg triggered and induced a high ovulation rate (100%) throughout the maturation cycle. This clear confirmation of DA inhibition in kutum by the pharmaceuticals, belonging to the third generation, is surprising. The detection of DA inhibition has important concepts for aquaculture, especially in captivity conditions, which often leads to a blockage of FOM and ovulation (Peter & Yu, 1997; Peter et al., 1991; Zohar et al., 2010; Yaron et al., 2003; Dufour et al., 1988). So, one of the most important DA receptor antagonists belonging to DA-D_2-like receptors, for instance, pimozide (Lin et al., 1988), domperidone (Kramer et al., 1993), metoclopramide (Koohilai et al., 2010) are related to the second generation antagonists (Maguire, 2002). OLZ, as the third generation antagonist, had higher occupancy of D_2-receptors and was introduced as a potent antagonist (Koohilai et al., 2017). However, OLZ becomes effective only at a dose of 5 mg, which crosses the conventional level of 100% D_2 occupancy. It seems that D_1-like receptors blockade is not necessary for reproduction of the species studied and D_2-receptors occupancy is required for maturational effectiveness. Indeed, the present study demonstrates higher doses of OLZ (10 mg) which had lower occupancy of D_2-receptor.

In conclusion, this study has revealed that clozapine and olanzapine were effective at optimum doses of 5 mg and 8 mg, respectively. This pharmaceutics were presented as the third generation of dopaminergic antagonists and belong to the D_2-

like receptor's antagonists. In fact, this is the first study to examine the effects of novel pharmaceutical compounds at different doses belonging to the dopaminergic and adrenergic systems, based on the fact that there was an inverse relationship between dopaminergic and adrenergic systems. Salbutamol and metoprolol belong to the β-adrenergic receptors, but there was an inverse relationship between them. The present observations on various catecholamines showed their important role in the reproductive cycle of fish.

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