

[Research]

## Effects of LHRH-A<sub>2</sub> and chlorpromazine (dopamine antagonists) on inducing spawning in Caspian Kutum, *Rutilus frisii kutum*, from the southwest of the Caspian Sea

M. Ahmadnezhad<sup>1\*</sup>, Sh. Oryan<sup>2</sup>, H. Hosseinzadeh Sahafi<sup>3</sup>, H. Khara<sup>4</sup>, M. Sattari<sup>5</sup>

1-Inland Water Aquaculture Research Center, P.O.Box 66, Bandar Anzali, Iran.

2-Biology Dept., Science Faculty, Kharazmi University, Tehran, Iran

3- Iranian Fisheries Research Organization, P.O.Box 14155-6116, Tehran, Iran

4- Dept. of Fisheries Sciences, Faculty of Natural Resources, Islamic Azad University of Lahijan, P.O. Box 1616, Iran.

5- Dept. of Fisheries, Faculty of Natural Resources, University of Guilan, Someh Sara, Iran.

\* Corresponding author's E-mail: m\_ahmadnezhad@yahoo.com

(Received: Apr. 3-2011, Accepted: Sept. 10-2011)

### ABSTRACT

Caspian kutum, *Rutilus frisii kutum*, is one of the most commercially important fish species in the Caspian Sea, but there are few reports about the endocrinology of induced spawning in this fish. In the present study, 54 individuals of female broodstocks of Kutum were studied for quantity and quality of propagation index, during March and April 2007. Hormone treatments include: LHRH-A<sub>2</sub> (1 µg kg<sup>-1</sup>bw; chlorpromazine (2.5 mg kg<sup>-1</sup>bw); LHRH-A<sub>2</sub> + chlorpromazine (1µg kg<sup>-1</sup>bw+2.5 mg kg<sup>-1</sup>bw); CPE as a positive control (1 mg kg<sup>-1</sup>bw); Physiological saline as a negative control; and normal brood stocks without injection. For histological examinations, ovarian samples of non-ovulated females were fixed in Bouin's fluid. The routine procedures of preparation of tissues were followed and the paraffin blocks were cut at 5-7 microns, stained with H&E and studied under light microscope. The results showed that the highest percentage of ovulated females belonged to group 1 without significant difference with positive control. There was a significant difference between ovulated females in the LHRH-A<sub>2</sub>, chlorpromazine and LHRH-A<sub>2</sub> + chlorpromazine treatments (P<0.05), while no significant differences in other propagation indices were found in any of the hormone treatments (P>0.05). Chlorpromazine could not be a potent dopamine antagonist and the microscopic observations of ovary in non-ovulated brood stocks showed that the oocytes in these treatments were in the final phase of IV stage.

**Keywords:** *Rutilus frisii kutum*, LHRH-A<sub>2</sub>, Chlorpromazine, Induced spawning.

### INTRODUCTION

Caspian Kutum, *Rutilus frisii kutum* belonged to the carp family (Nelson 1976) is one of the most commercially important fish species and is native to the southern part of the Caspian Sea. The distribution of kutum is limited from the mouth of Volga River in the north Caspian Sea to the Gulf of Aster-Abad in the southwest of this sea. So, aggregation of its population is related to Iranian water bodies (Abdoli 1999). Declining population of Kutum in relation to some ecological and biological factors such as loss of spawning grounds, uncontrolled fishing and overfishing, as well as discharge of physicochemical pollutants to rivers in the past three decades induced the Iranian Fisheries Organization to enhance semi

artificial and artificial propagation of Kutum for the purpose of releasing its larvae into the rivers of the Caspian Sea. For this reason every year, kutum brood stocks are caught from rivers and estuaries and then are reproduced by semi-artificial methods. However, because the substrate of Sefidrud River is full of clay and mud and also because of water pollution, the kutum should migrate upstream to seek spawning grounds. Unfortunately, because adult Kutum are caught illegally by numerous fishermen, fisheries experts have to capture immature brood stocks in delta of rivers especially in Sefidrud River and transport them to the reproduction and propagation centers. Nowadays these centers use carp pituitary extract to induce ovulation in

Kutum brood stocks, which is expensive and also scarce. So, for inducing ovulation in different fish species, various forms of gonadotropin-releasing hormone (GnRH) and its agonists (GnRH<sub>a</sub>) were used. Dopamine receptor antagonists are added and used to strengthen the response to injection of a GnRH peptide in fish reproduction process (Peter *et al.* 1988). The benefits of using of gonadotropin releasing hormone (GnRH) are that it releases pituitary gonadotropin in fish with no side effects on the reproduction period, the incidence of hormone receptors in fish immune responses, ease of access, usage and also the prices of these ingredients is a priority (Yaron 1995; Donaldson 1996; Peter & Yu 1997). GnRH<sub>a</sub> effect of dopamine antagonists on ovulation depends on the material and its concentration, environment and fish species (Zohar & Mylonas 2001). Therefore it is necessary to test the response of any species to the combination of GnRH and dopamine antagonists. The effectiveness of synthetic LHRH (Luteinizing Hormone Releasing Hormone) in inducing spawning behavior was proved in 1975, LHRH-A (Luteinizing Hormone Releasing Hormone Analogue) with the high effects and reduced cost was produced (Nazari 1996). Successful use of agonists GnRH alone or in combination with an antagonist of dopamine in inducing spawning in different species has been reported by: Yaron 1995; Peter *et al.* 1988; Zohar 1989; Zohar & Myloas 2001; Szabo *et al.* 2002. In Iran studies on GnRH alone or in combination with dopamine antagonists on rainbow trout, *Oncorhynchus mykiss* (Peykan Heyrati *et al.* 2001; Dorafshan *et al.* 2002), common carp, *C. carpio* (Rasekhifar 1998; Dorafshan *et al.* 2003; Ghobadi 2004) and silver carp, *Hypophthalmichthys molitrix* (Kashani Sabet *et al.* 2004) were carried out. In this study, the hormone LHRH-A<sub>2</sub>, a potent GnRH analog, and chlorpromazine, dopamine receptor antagonists have been used. LHRH-A<sub>2</sub> hormone induces hormone synthesis and has immediate effect as a stimulus on the secretion of hormones in sex glands of bony fish. LHRH-A<sub>2</sub> has biological effectiveness according to fish species and it is 100 times more potent than LHRH. LHRH easily decomposes by protease and disappears. Chlorpromazine, [3- (2-Chlorophenothiazin-10-yl) propyldimethylamine hydrochloride],

is an antagonist that acts on different synaptic D2 receptors. The objectives of this study are: a) to induce maturation of Kutum and ovulation using hormonal injection; b) to investigate whether adding a dopamine antagonist is required for induction of ovulation in Kutum; and c) to evaluate the effect of hormonal treatments on the quality of propagation through various quantitative and qualitative indicators.

#### MATERIALS and METHODS

In the present study 72 female kutum (600 to 900 g mean=742 g SD=164g) were caught from the river mouth and estuary of the Sefid Rud River during their migration season (March - May 2007) and were examined at the Fish Rearing, Propagation and Restocking Center of Shahid Ansari in Guilan province, Iran. Tests were carried out in two phases, first in a pilot phase to determine the optimal dose of the hormone LHRH-A<sub>2</sub> and in second phase with chlorpromazine. In the pilot phase, 18 female of kutum were transferred to six tanks (three brood stocks per tank each tank with 500 l capacity). The injection treatments were as follows: First the three groups received 0.5, 1 and 2 mg LHRH-A<sub>2</sub> per kg of body weight respectively. Second, the three groups were injected with 2.5, 5 and 7.5 mg chlorpromazine per kilogram of body weight, respectively. After defining the desired dose (based on the percentage of accountable eggs, 1µg kg<sup>-1</sup> bw for LHRH-A<sub>2</sub> and 2.5 mg kg<sup>-1</sup> bw for chlorpromazine), the main test phase was started with 54 brood stocks in 6 different treatment groups (nine fish in each treatment). The treatments were as follows: 1- LHRH-A<sub>2</sub> (1µg kg<sup>-1</sup> bw), 2- chlorpromazine (2.5 mg kg<sup>-1</sup> bw), 3- both chlorpromazine and LHRH-A<sub>2</sub> (2.5 mg kg<sup>-1</sup> bw + 1µg kg<sup>-1</sup> bw), 4- injection of pituitary extract (positive control) (1mg kg<sup>-1</sup> bw), 5- 1 ml of normal saline solution 0.7% per kg of body weight and 6- normal brood stocks with no injection.

In both phases of experiment, fish weight was measured and they were injected according to body weight. Fish were placed in qualified fiberglass tanks with water temperature at 10-13 °C. The maximum volume of 1 ml aliquots were injected intramuscularly in a single dose according to body weight (kg). Injected brood stocks

were observed during the first 24 hours and then were observed every ten hours. At each observation, based on the relaxation of abdomen, stripping process was started in mature brood stocks. After weighing the extracting eggs, the gametes of each female were crossed fertilized with sperm of three males. After hardening of eggs and removal of adhesion, eggs were transferred into trays and then brood stock characteristics and the kinds of treatments were recorded on each tray. In the pilot phase, only response dose of each experimental group in the main treatments was considered. In the main phase of experiment, these parameters were evaluated:

Ovulated female (%) = number of stripped females / number of injected females  $\times$  100

Ovulation Index (OI %) = Weight of extracted eggs (g)/total remaining ovary and extracted eggs weight  $\times$  100 (Szabo *et al.* 2002).

Latency Period= mean time between injection and the time of ovulation (h) (Drori *et al.* 1994).

Percentage of extracted egg weight per body weight= weight of eggs produced by each broodstock (g) / brood stock body weight (g)

Degree - hour of sexual maturity= duration of injection time to sexual maturity (h)  $\times$  the average temperature

Fertilization success (%) = the rate of fertilized eggs in Gastrulating stage / the total eggs sampled  $\times$  100 (Razavi Sayad 1995)

Degree - day of hatching= the time during fertilization until hatching stage in day  $\times$  the average temperature

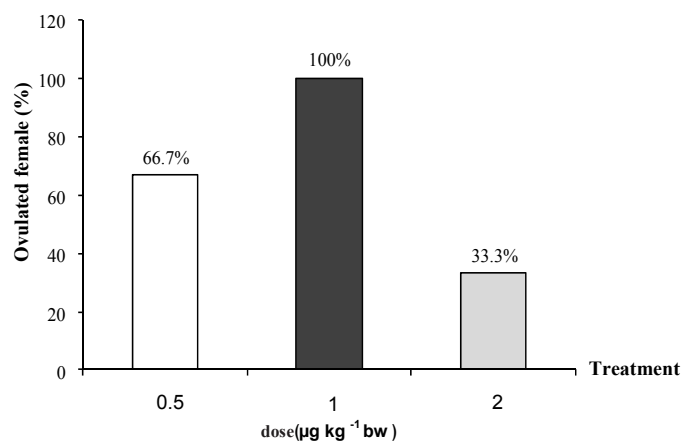
Hatching rate (%) = number of larvae with yolk sacs /the total number of fertilized eggs  $\times$  100.

For histological examination samples of broodstock ovary which did not respond to injections after 72 hours were removed and fixed in Bouin's fluid for 48 hours, washed and then rinsed with 70% ethanol, dehydrated in an ascending series of ethanol for embedding in Paraffin. Then transverse and longitudinal sections of 5  $\mu$ m diameters were cut on a Leitz Wetzlar microtome and were transferred onto glass slides (Poosti & Marvasti, 1999). The sections were then stained with standard haematoxylin and eosin (H&E) stain (Kazemi & Bahmani 1998). Stained samples were observed by microscope on a Nikon digital optical monitor and then photographed. The statistical analysis of all data were carried out using Independent sample one-way analysis of variance (ANOVA) test and Tukey mean comparison test with a confidence of 95% ( $P < 0.05$ ). Percentage of ovulated female was analyzed by  $\chi^2$  test (Chi-square test) (Szabo *et al.*, 2002). All statistical analyses were carried out using SPSS 13 for windows software package and Excel for graphing.

## RESULTS

### Initial test results to determine the optimal dose of LHRH-A<sub>2</sub> and Chlorpromazine

All the broodstocks ovulated by injection of 1  $\mu$ g kg<sup>-1</sup>bw of LHRH-A<sub>2</sub> were significantly different from other groups ( $P < 0.05$ ) (Fig. 1). So, 1  $\mu$ g kg<sup>-1</sup> LHRH-A<sub>2</sub> was selected (Fig. 1).



**Fig 1.** Comparison of percentage of ovulated females in three treatment groups for determination of LHRH-A<sub>2</sub> optimal dose.

In chlorpromazine treatment groups, no fish ovulated with 7.5 mg kg<sup>-1</sup> bw but the proportion of ovulated females in two other groups were 66.7%. Hence 2.5 mg kg<sup>-1</sup> bw

was selected because a lower dose with an equal proportion of ovulated female is more appropriate in terms of low cost and lower side effects (Fig 2).

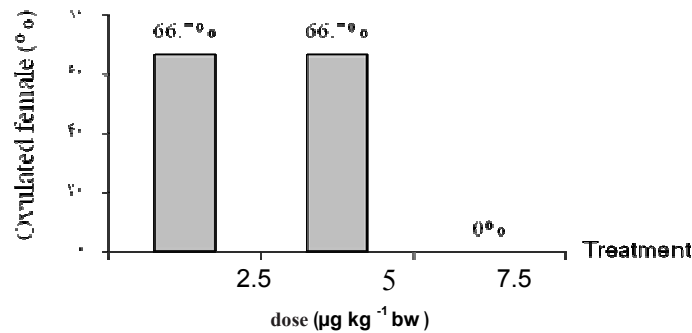


Fig 2. Comparison of percentage respond between treatments in percent accountable chlorpromazine

#### Test results in original research treatments

No fish ovulated in the negative control (saline 0.7%) treatment and non injected matured group. In the positive control (pituitary extract) 77.78 % of fish ovulated. Among hormone treatments, the highest percentage of ovulated females belonged to LHRH-A<sub>2</sub> group (1µg kg<sup>-1</sup>bw) (66.7 %) and the lowest was observed in chlorpromazine group (2.5 mg kg<sup>-1</sup>bw) (22.2 %). There were no significant differences between LHRH-A<sub>2</sub> group and the positive control, while these two groups showed significant differences with the chlorpromazine group and its combination with LHRH-A<sub>2</sub> (P<0.05).

The highest value of degree - day of maturity and latency period was observed in LHRH-A<sub>2</sub> group alone, while the lowest value of these indices belonged to the

group of LHRH-A<sub>2</sub> combined with chlorpromazine.

Ovulation index was in the range of 72.8 - 81.1, and the highest value of OI was observed in the positive control. Also, egg weight / body weight ratio (%) and of degree - day of hatching in the positive control group was the highest. Chlorpromazine group had the highest percentage of fertilization success and hatching rate. However, no significant differences in these propagation indices were found in any of the hormone treatments (P>0.05). Fertilization success in treated fish was in the range of 65.4%-98.1% and was within the normal range of hatchery practice for Kutum. There were no significant differences in fertilization success among groups (P>0.05) (Table 1).

Table 1. The effect of different hormone treatments on ovulated female (%) and optimum propagation index of *Rutilus frisii kutum*

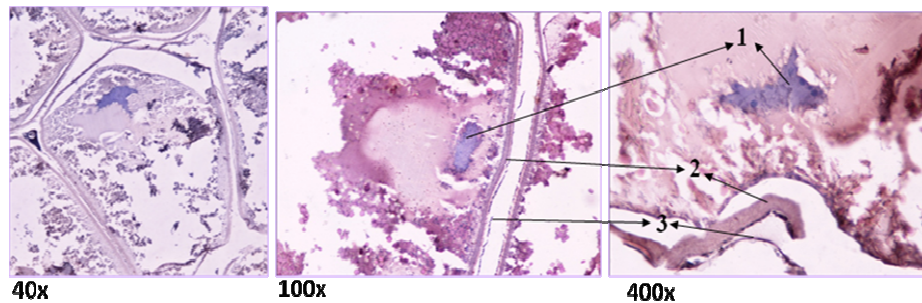
Groups	Treatment	Dosage*	Ovulated female (%)	degree - day of sexual maturity	Latency period (h)	ovulation index (%)	egg weight percent, relative to body weight	Fertilization success (%)	Hatching rate (%)	degree - day of hatching
1	LHRH-A <sub>2</sub>	1	66.7% <sup>a</sup>	480.2±118.7 <sup>a</sup>	39.6±9.6 <sup>a</sup>	74.6±8.5 <sup>a</sup>	12.5±1.4 <sup>a</sup>	78.8±15.9 <sup>a</sup>	36.5±11.9 <sup>a</sup>	139.5±4.5 <sup>a</sup>
2	Chlorpromazine	2.5	22.2% <sup>b</sup>	405.2±124.6 <sup>a</sup>	33.3±10.3 <sup>a</sup>	79.2±5.3 <sup>a</sup>	11.9±2.4 <sup>a</sup>	98.1±0.2 <sup>a</sup>	50.1±2.0 <sup>a</sup>	131.0±0.0 <sup>a</sup>
3	Chlorpromazine + LHRH-A <sub>2</sub>	2.5+1	33.3% <sup>b</sup>	388.7±109.0 <sup>a</sup>	31.8±8.3 <sup>a</sup>	72.8±16.3 <sup>a</sup>	12.2±3.8 <sup>a</sup>	65.4±32.7 <sup>a</sup>	31.2±23.3 <sup>a</sup>	137.7±8.7 <sup>a</sup>
4	injection of pituitary extract (positive control)	1mg	77.8% <sup>a</sup>	416.3±54.1 <sup>a</sup>	34.9±4.7 <sup>a</sup>	81.1±2.9 <sup>a</sup>	13.4±2.3 <sup>a</sup>	84.6±8.9 <sup>a</sup>	35.8±9.4 <sup>a</sup>	139.6±4.3 <sup>a</sup>
5	Saline (0.7% NaCl)	-	0	-	-	-	-	-	-	-

Histological examinations showed that the ovarian samples of non- ovulated females

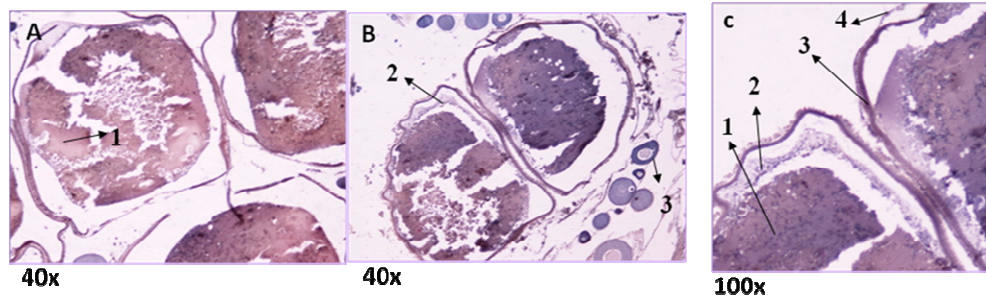
in chlorpromazine group (2.5mg kg<sup>-1</sup> bw), chlorpromazine + LHRH-A<sub>2</sub> (2.5 mg kg<sup>-1</sup>

bw + 1 $\mu$ g kg<sup>-1</sup> bw), negative control (0.7% saline) and brood stocks group without injection were in the final phase of IV stage (Fig 3). The ovarian samples of non-

ovulated females in the positive control (pituitary extract 1mg kg<sup>-1</sup> bw) and LHRH-A<sub>2</sub> group (1 $\mu$ g kg<sup>-1</sup> bw) were in the early stage V of maturation (Fig. 4).



**Fig 3.** Histological section of a non-ovulated female gonad of the kutum, *Rutilus frisii kutum*, in groups 5 and 6. Oocytes are in late phase of stage IV of sexual maturation. The germinal vesicle nearer to oocyte membrane, nucleuse is scalloped and nucleolus is distributed in nucleus. 1. Germinal vesicle, 2. Zona radiata, 4. Theca.



**Fig 4.** Histological section of a non-ovulated female gonad of kutum, *Rutilus frisii kutum*, in LHRH-A<sub>2</sub> treatment. 1A, 1C. Yolk granules are compressed. 2B, 2C. Membrane yolk vesicles pressed together. 3B. Oocyte in stage II. 3C. Thick Zona radiata. 4C. Follicular layer and Theca.

## DISCUSSION

In the negative control treatment (normal saline, 0.7%) and the broodstocks group without injection, no fish spawned. It suggests that for induction of broodstocks to spawn, the hormonal treatment is required. Carp pituitary extract as a positive control, with dose of 1 mg / kg body weight in females, could induce spawning. In the present study, LHRH-A<sub>2</sub> alone induced ovulation in Kutum, but although the percentage of the eligible ovulation value in this treatment (66.7%) was lower than positive control with pituitary extract (77.8 %), there was no significant difference ( $P > 0.05$ ). These results show that LHRH-A<sub>2</sub> is an active and effective substance for inducing final maturation of Kutum brood stocks, and possibly the use of this hormone in the final stages of maturation accelerates the completion of germinal vesicle migration and its breakdown (GVBD) and causes

spawning of brood stocks to occur soon. This result, compared with the results of induction of spawning by GnRH-A is consistent with a number of reports about other bony fishes. There are many reports on the effects of different GnRH-A substances on oocyte maturation, ovulation and spawning in some fish species such as Coho salmon, *Oncorhynchus kisutch* (Breton et al. 1990); big head carp, *Aristichthys nobilis* (Fermin 1991); bream, *Abramis brama* (Glubokov et al. 1991); tench, *Tinca tinca* (Kouril et al. 1986), milk fish, *Chanos chanos* (Lee et al. 1989), *Paramisguruns dadryanus* (Lin et al. 1988), common carp, *Cyprinus carpio* (Nandeesh et al. 1989) and crucian carp, *Carrassius auratus* (Peter et al. 1985). Percentage of ovulated females in chlorpromazine treatment (22.2 %) was lower than that in LHRH-A<sub>2</sub> treatment (66.7 %) and there were statistically significant differences ( $P < 0.05$ ) between

them. This suggests that chlorpromazine could not be a potent dopamine antagonist in hypothalamus pituitary gonad axis of Kutum, so it seems that the antagonist cannot be suitable for inhibitory effects of dopamine on GTH (Gonadotropine hormone) release. Injection of chlorpromazine alone and in combined with LHRH-A<sub>2</sub> induced ovulation in Kutum but the ovulated female had obviously lower response than LHRH-A<sub>2</sub> and CPE-treated fish. These results showed that dopamine (DA) has inhibitory effect on GTH secretion in Kutum but its effect is not considerable. So, involvement and influence of dopamine receptors on maturation of females of Kutum does not have dominant and significant advantages. The results show that blocking effect of dopamine on GTH secretion in Kutum is of minor importance and dominance than in other cyprinids, such as *Cyprinus carpio* (Arabaci *et al.* 2004; Dorafshan *et al.* 2003; Drori *et al.* 1994) and *Chondrostoma nasus* (Szabo *et al.* 2002).

Inhibition of dopamine on GTH release varies among different fish species and many fish families such as cyprinids and African catfish, *Clarias gariepinus* (Mylonas & Zohar 2001), but there is no report about inhibition effects in most salmonids such as Coho salmon, *Oncorhynchus kisutch* (Van Der Kraak *et al.* 1986) and commercially valuable marine fish (Mylonas & Zohar, 2001). Also it seems that the significance of DA inhibition changes during the reproduction cycle and is usually minimal during the spawning season (Linard *et al.*, 1995). In some species such as *Parabramis pekinensis* (Lin *et al.* 1986), *Paramisgurnus dabryanus* (Lin *et al.* 1988) and tilapia hybrids, *Tilapia nilotica* × *Tilapia undulates* (Gissis *et al.* 1991) dopamine does not have important deterrent effects on releasing of GTH and also GnRH alone can induce ovulation, but when the combination of GnRH<sub>a</sub> and dopamine antagonists is applied, the dose of GnRH<sub>a</sub> may strengthen or reduce its effect. So it suggests that Kutum could be classified in this group of fish.

Histological examinations of gonad maturation in brood stocks treatment with no injection and those which did not respond to multiple injections, suggest that ovaries are in the final stages of ovarian

maturation and sexual maturity and this confirms that hormonal treatments of broodstocks respond to injection and induce ovulation.

The females responding to chlorpromazine had a lower time span between injection and ovulation as compared to other treatments. This effect is probably related to the low half life of chlorpromazine (8 to 35 hours) (Dollery 1991). The results on index of injection-to-ovulation period of Kutum in this study showed that this index in *Heteropneustes fossilis* (Alok *et al.* 1993), common carp, *Cyprinus carpio* (Yaron, 1995; Dorafshan *et al.* 2003; Arabci *et al.* 2004); silver carp, *Hypophthalmichthys molitrix* (Kashani Sabet 2004) and other cultured cyprinids (Billard 1990) was most probably related to temperature in the spawning season. The temperature for spawning of these fish is less (about 7 to 15°C) than that of Kutum. A very good indicator for evaluating the effectiveness of hormonal treatment is ovulation index (Szabo *et al.*, 2002). In the present experiment, the lowest number related to ovulation index was belonged to chlorpromazine (2.5 mg kg<sup>-1</sup> bw) + LHRH-A<sub>2</sub> (1µg kg<sup>-1</sup> bw) (72.8%). However, there was no significant difference between this group and the other ones. So, all of the treatment groups have an acceptable ovulation index. According to the average percentage of egg weight to body weight, the use of LHRH<sub>a</sub> or dopamine antagonists have not made any significant changes in the rate of egg maturation. It should be noted that these hormones and drugs were only used in the final stages of maturation in brood stocks. So, it cannot affect the number of eggs produced by each brood stocks, because the amounts of produced eggs in each sexual rhythm can be established over a long time and are related to other factors such as genetic, hormones, environment, nutrition that can affect and control these process (Zohar, 1989). Haraldson *et al.* (1993) during a study on Arctic char, *Salvelinus alpinus* suggested that after induction of spawning no significant difference was observed between the weight of extracted eggs and the remaining eggs in the abdominal cavity of fish treated with the control group. Similar results have been reported in *Cynoscion nebulosus* by other authors (Peter

& Yu 1997; Erdahl & Clain 1987).

All of the qualitative and quantitative factors on reproduction in this study, such as fertilization rate and hatching percentage and hatching degrees - day also between treatment groups were not significantly different. Various hormonal treatments had no negative impact on the survival of eggs; therefore various hormonal treatments in this experiment had no significant impact on the quality of reproduction. A similar result was obtained on the fertilization rate of common carp (Drori *et al.* 1994; Kulikovskiy *et al.* 1996; Dorafshan *et al.* 2003). They showed that use of GnRH alone or in combination with dopamine antagonists in pre ovulated fish eggs had no effect on the quality of them (Zohar & Mylonas, 2001). In addition, Brzuska and Adamek (1999) during a study on European catfish showed no significant impact of ovulation stimulators on the qualitative and quantitative features of eggs. Results of the present study suggest the deterrent effect of dopamine on GTH secretion in Kutum and showed that sGnRH alone can induce ovulation in Kutum. Using LHRH-A<sub>2</sub> (1µg kg<sup>-1</sup> bw) could be an effective way to induce maturation and ovulation in Kutum and is a substitute for pituitary extract in hatcheries, because despite having a similar effect on spawning, it is cheaper and needs a lower dose.

#### ACKNOWLEDGMENT

We are very grateful to from Head of Iran Fisheries Organization Dr Nezami, then director general of Guilan Fisheries Mr. Soheil Mohammadi, Head of Inland Water Aquaculture Research Institute (Bandar Anzali) Mr. Khani Pour and all of staff in the Department of the Institute of Aquaculture. Our thanks are also due to the President and staff of the Fish Rearing, Propagation and Restocking Center, Shahid Ansari Rasht.

#### REFERENCES

- Abdoli, A. (1999) Inland Water Fishes of Iran. *Nature and Wildlife Museum Publications*. 377 p, 115-120.
- Alok, D., Krishnan, T., Talwar, G. P. and Gary, L.C. (1993) Induced spawning of catfish, *Heteropneustes fossilis* (Bloch), Using D-Lys<sup>6</sup>, Salmon gonadotropin - releasing hormone analog. *Aquaculture*. **115**, 159-167.
- Arbaci, M., Cegirgan, H., Sari, M. (2004) Induction of ovulation in ornamental common carp (Koi, *Cyprinus carpio* L.) using LHRHa ([D-Ser9 (tBu6), Pro9-Net]-LHRH) combined with haloperidol and carp pituitary extract. *Aquaculture Research*. **35**, 10-14.
- Billard, R. (1990) The major carps and other cyprinids. In: Production of Aquatic Animals (Fishes) (ed. by C.E. Nash), pp. 21-55. *Elsevier science publication*, Amsterdam, the Netherlands Biswas, S.P.1993. Manual of methods in fish biology. South Asian publisher. New Dehli, pp. 190.
- Breton, B., Weil, C., Sambron, E., Zohar, Y. (1990) Effects of acute versus sustained administration of GnRH-a on GTH release and ovulation in the rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*. **91**, 373-383.
- Brzuska, E., Adamek, J. (1999) Artificial spawning of European catfish, *Silurus glanis* L.: stimulation of ovulation using LHRH-a, Ovaprim and carp pituitary extract. *Aquaculture Research*. **30**(1), 59-64
- Dollery, C. (1991) Therapeutic Drugs. Volume 1. Edinburgh: Churchill Livingstone, 201-206.
- Donaldson, E.M. (1996) Manipulation of reproduction in farmed fish. *Animal Reproduction science* **42**, 381-392.
- Dorafshan, S., Majazi Amiri, B., Hajizadeh, A., Mostafavi, H. Paykan Heyrati, F. (2002) Induced proliferation of female rainbow trout *Oncorhynchus mykiss* using the synthetic hormone GnRH<sub>a</sub>. *Iranian Journal of Fisheries*. **3**, 23-38.
- Dorafshan, S., Mostafavi, H., Mojazi Amiri, B. (2003) Induction of spawning in common carp (*Cyprinus carpio*), using pituitary extract and GnRH analogue in combination with domperidone. *Iranian Journal of Biotechnology*. **1**, 213-217.
- Drori, S., Ofir, M., Sivan, B.L. Yaron, Z. (1994) Spawning induction in common carp (*Cyprinus carpio*) using pituitary extract or GnRH super active analogue combined with methoclopramide: analysis of hormone profile, progress of oocyte maturation and dependence on temperature. *Aquaculture*. **119**:393-407.



- Erdahl, D.J., McClain, J. (1987) Effect of LHRH analogue treatment on egg maturation (Ovulation) in lake trout brook stock. *Progressive fish-culturist*. **49**, 276-279
- Fermin, C.A. (1991) LHRH-a and domperidon- induced oocyte maturation and ovulation in bighead carp, *Aristichthys nobilis*. *Aquaculture*. **93**, 87-94.
- Gissis, A., Levavi-sivan, B., Rubinp-kedem, H., ofir, M., Yaron, Z. (1991) The effect of gonadotropin releasing hormone superlative analogue and dopamine antagonists on gonadotropin level and ovulation in tilapia hybrids. *J. Aquacultu - Bamidgeh* **43**, 123-136.
- Glubokov, A.I., Motloch, N.N., Sedova, M.A. (1991) Effect of synthetic LHRH analogue and dopamine antagonists on the maturation of bream, *Abramis brama*. *Aquaculture* **95**, 373-377.
- Ghobadi, Sh. (2004) Comparative study on effects of LHRH and dopamine antagonists (Pimozide) sexual maturation in common carp maturation (*Cyprinus carpio*). *MSc Thesis. Islamic Azad University of Lahijan, Iran*. 60p.
- Haraldson, H., Sveinsson, T., Skulason. S. (1993) Effects of LHRH-a treatments upon the timing of ovulation & upon egg and offspring quality in arctic charr, *Salvelinus alpinus*. *Aquaculture & Fisheries Management* **24**, 145-150.
- Kashani Sabet, A. R., Oryan, Sh., Bahmani, M. (2004) ovulation Induction in silver carp phytophage, *Hypophthalmichthys molitrix* by using of LHRH-A and its combination with dopamine antagonists (Metacloperamid and Dompridone). *Journal of Fisheries of Iran*. **3**, 129-144.
- Kazemi, R., Bahmani, M. (1998) Instructions for preparing and staining tissues for histological studies. *International Institute of sturgeon Dr Dadman*. 16 p.
- Kouril, j., Barth, T., Hamackova, J., Flegel, M. (1986) Induced ovulation in tench, *Tinca L.* various LHRH synthetic analogues: Effect of site of administration and temperature. *Aquaculture* **54**: 37-44.
- Kulikovsky, Z., Martin, F.J.B., Yaron, Z. (1996) A comparison of two spawning inducing agent for common carp. *The Israeli Journal of Aquaculture, Bamidgeh* **48**, 108-111.
- Lee, C. S., Tamaru, C. S., Kelley, C. S., Banno, J.E. (1986) Induced spawning of milkfish, *Chanos chanos*, by a single application of LHRH - analogue. *Aquaculture* **58**, 87-98.
- Lin, H.R., Chun, p., Van Der Kraak, G., Peter, R.E., Breton, B. (1986) Effects of [D-Ala<sup>6</sup>, Pro<sup>9</sup>- Net] - LHRH and catecholaminergic drugs on gonadotropin secretion and ovulation in the Chinese loach, *Paramisgurnus dabryanus*, *General Comparative Endocrinology*. **64**, 389-395.
- Lin, H.R., Van Der Kraak, G., Zho, X-J., Liang, J.Y., Peter, R.E., Rivier, J.E., Vale, W.W. (1988) Effects of [D-Arg<sup>6</sup> Trp<sup>7</sup> Leu<sup>8</sup> Pro<sup>9</sup> Net]-luteinizing hormone-releasing hormone (sGnRH-A), and [D-Ala<sup>6</sup> Pro<sup>9</sup> NET]-luteinizing hormone-releasing hormone (LHRH-A), in combination with pimozide or domperidone, on gonadotropin release and ovulation in the Chinese loach and Common carp. *General Comparative Endocrinology*. **69**, 31-40.
- Linard, B., Bennani, S., Saligaut, C. (1995) Involvement of estradiol in a catecholamine inhibitory tone of gonadotropin release in the rainbow trout (*Oncorhynchus mykiss*). *General Comparative Endocrinology*. **99**, 192-196.
- Mylonas, C.C., Zohar, Y. (2001) Use of GnRHa-delivery systems for the control of reproduction in fish. *Reviews in Fish Biology and Fisheries* **10**, 463-491.
- Nandeesh, M.C., Das, S.K., Nathaniel, E., Verghese, T.J., shetty, H.P.C. (1989) Ovaprim a new drug for induced breeding of carps. *Fishing chimes*, 13-15.
- Nazari, R. M. (1996) biology and artificial propagation of Silver carp, *Hypophthalmichthys molitrix*. *Shilat Department in Bandar anzali, Iran*. 94 p.
- Nelson, J.S. (1976) Fishes of the world. *New York, John wiley and Sons*.
- Paykan Heyrati, F., Mostafavi, H., Mojazi Amiri, B., Hajizadeh, A., Dorafshan, S. (2001) Induced spermiation of rainbow trout (*Oncorhynchus mykiss*) using a GnRH analogue. *Iranian Journal of Fisheries Science* **3**, 95-108
- Peter, R.E., Nahorniak, C.S., Sokolowsha, M., Chang, J.P., Rivier, J.E, Vale, W.W., King, J.A., Millar, R.P. (1985) Structure-activity relationship of



- mammalian, chicken and Salmon gonadotropin releasing hormone in vivo in goldfish. *General Comparative Endocrinology* **58**, 231-242.
- Peter, R.E., Lin, H.R., Van Der Kraak, G. (1988) Induced ovulation and spawning of cultured fresh water fish in china: advances in application of GnRH analogues and dopamine antagonists. *Aquaculture* **74**, 1-10.
- Peter, R.E., Yu, K.L. (1997) Neuroendocrine regulation of ovulation in fishes: basic and applied aspects. *Rev. fish Biology. Fish* **7**, 173-197.
- Poosti, A.S., Marvdasti, S. (1999) Atlas of fish histology (normal and pathological forms), *Tehran University Press*. 328 p.
- Rasekhifar, Kh. (1998) Effects of application of Baclofen (agonist receptor GABA - B) alone and in combination with LHRH - A Metacloperamid and the release of GTH-I in carp (*Cyprinus carpio* L.). MSc Thesis. *University of Tarbiat Moalem, Tehran, Iran*. 120 p.
- Razavi Sayad, B. (1995) Kutum fish. *Fisheries Research Institute, Bandar anzali, Iran*. 164 p.
- Szabo, T., Medgyasszay, C., Horvath, L. (2002) Ovulation induction in nase (*Chondrosoma nasus*, Cyprinidae) using pituitary extract or GnRH analogue combined with domperidone. *Aquaculture* **203**, 389-395.
- Van Der Kraak G., Donaldson E.M., Chang J.P. (1986) Dopamine involvement in the regulation of gonadotropin secretion in Coho Salmon. *Canadian Journal of Zoology*, **64**, 1245-1248.
- Yaron, Z. (1995) Endocrine control of gametogenesis and spawning induction in the carp. *Aquaculture* **129**, 49-73.
- Zohar, Y. (1989) Fish reproduction, its physiology and artificial manipulation. In: *Fish Culture in Warm Water Systems, Problems & Trends* (ed. By M.C. Shilo & S.H. Sargi), pp.65-119. CRC Press, Boca Raton, FL, USA.
- Zohar, Y., Mylonas, C.C. (2001) Endocrine manipulation of spawning induction in cultured fish from hormone to gene. *Aquaculture* **197**, 99-139.

## تاثیر LHRH-A<sub>2</sub> و کلرپرومازین (آنتاگونیست دوپامین) بر القای تخم ریزی ماهی سفید *Rutilus frisii kutum* در جنوب غربی دریای خزر

م. احمدنژاد، ش. عریان، ه. حسین زاده صحافی، ح. خارا، م. ستاری

### چکیده

در این تحقیق از 54 مولد ماده ماهی سفید در زمان تکثیر (اسفند 1385 تا اردیبهشت 1386) بمنظور بررسی اثرات استفاده از هورمون LHRH-A<sub>2</sub> و داروی کلرپرومازین بر روی برخی از شاخص های کمی و کیفی تکثیر، استفاده گردید. تیمارها بصورت: 1- LHRH-A<sub>2</sub> ( $1 \mu\text{g kg}^{-1} \text{ bw}$ )، 2- کلرپرومازین ( $\text{mg kg}^{-1} \text{ bw}$ )، 3- ترکیب کلرپرومازین و LHRH-A<sub>2</sub> ( $1 \mu\text{g kg}^{-1} \text{ bw} + 2/5 \text{ mg kg}^{-1} \text{ bw}$ )، 4- تزریق عصاره هیپوفیز (شاهد مثبت) ( $1 \text{ mg kg}^{-1} \text{ bw}$ )، 5- محلول نرمال سالین 0/7 درصد به میزان 1 سی سی به ازای هر کیلوگرم وزن بدن ماهی (شاهد منفی) و 6- گروه ماهی بدون تزریق، انتخاب گردیدند. تخمدان مولدینی که به تزریق پاسخ مثبت ندادند با استفاده از روش بافت شناسی کلاسیک و رنگ آمیزی هماتوکسیلین و ائوزین مورد مطالعه میکروسکوپی قرار گرفتند. نتایج نشان داد که بیشترین درصد جوابدهی مولدین ماده پس از گروه شاهد مثبت، به تیمار LHRH-A<sub>2</sub> تعلق داشت که با سایر گروهها اختلاف معنی دار داشتند ( $P < 0.05$ ). از نظر سایر شاخص های کمی و کیفی تکثیر بین گروهها هیچ اختلاف معنی داری وجود نداشت ( $P > 0.05$ ). بنظر می رسد که کلرپرومازین نمی تواند بعنوان یک آنتاگونیست دوپامینی قوی در این مولدین عمل نماید. در مشاهدات میکروسکوپی از بافت تخمدان اغلب ماهیانی که در تیمارهای مختلف به تزریق پاسخ نداده بودند مشخص شد که تخم ها در اواخر مرحله چهار رسیدگی جنسی قرار داشتند.