JOURNAL OF CURRENT RESEARCH IN SCIENCE (ISSN 2322-5009)
CODEN (USA): JCRSDJ
2013, Vol. 1, No. 4, pp:192-198
Available at www.jcrs010.com

ORIGINAL ARTICLE

HORMONAL INDUCTION AND SYNCHRONIZATION OF OVULATION IN ENDANGERED CASPIAN BROWN TROUT (*SALMO TRUTTA CASPIUS* KESSLER, 1877) AND ITS EFFECTS ON EGG QUALITY

Seyed Ehsan Mousavi
Department of Fishery, Qaemshahr branch, Islamic Azad University, Qaemshahr, Iran

ABSTRACT: The aim of this study was to evaluate the effects of different hormones to induce and synchronizing of ovulation in an early spawning of *Salmo trutta caspius*. Fish were selected in the beginning of the spawning season and were allocated into five groups and were treated intraperitoneally; (I) 0.5 ml of sGnRHa in two injections (GnRHa–Dom, GnRH-1), (II) 0.5 ml propylene glycol-dissolved mGnRHa in two injections (GnRHa–Met, GnRHa-2), (III) 0.5 ml propylene glycol-dissolved [D-Trp6-mGnRH] in two injections (GnRHa–Met, GnRHa-3) and (IV) 0.5 ml propylene glycol-dissolved sGnRHa in two injections (GnRHa– Met, GnRHa-4) and (V) 0.5 ml propylene glycol in two injections (control group). The GnRHa and dopamine antagonist doses in all hormone treatments were 20 µg kg-1 and 10 mg kg-1 body weight respectively. In each injection, fish received half dose of hormone. All treatment groups received two injections four days apart. All fish in treatment I & II groups had 100% ovulated within 15 & 14 days after treatment, respectively. While 50% and 83.33% of the fish in the treatment III & IV ovulated within 18 to 21 days after treatment respectively. The control group had only 33.3 % after 31 days. Fish injected with the GnRHa had a mean time ovulation of 13 days, compared with 24 days for control treated fish. None of the treatments caused any pre- or post-spawning mortality in the broodstock. Fertilization, eyeing and hatching percentages of the produced progeny were normal in all the treatment groups and did not differ significantly among them ($p>0.05$). In conclusion, all GnRHa-DA treatments were effective in advancing the onset of ovulation, synchronizing the ovulation of the treated fish and shortening the egg collection period, relative to the control without affecting broodstock survival and egg quality.

KEYWORDS: GnRHa, Dopamine antagonist, Induction of ovulation, *Salmo trutta caspius.*

INTRODUCTION

The Caspian brown trout, *Salmo trutta caspius,* one of the most important fish of Caspian Sea belongs to Salmonidae, and is an endangered anadromous fish which such as sturgeon fish, has been artificially reproduced for restocking purpose (Yousefian *et al.,* 2010; Mousavi *et al.,* 2011). Cultivated stocks have been reared for enhancement and protection of wild populations and maintenance of this species depends on stocked fish originating from aquaculture systems**.** The acceleration of final oocyte maturation is important in this fish, due to the economics of broodstock management. In most fish species, however, the need to collect the eggs by stripping is a serious limitation, as the time of ovulation must be predicted with accuracy, as over-ripening may take place in minutes or hours after ovulation (Mylonas *et al.,* 2010; Yousefian and Mousavi, 2011a; Yousefian and Mousavi, 2011b). While in salmonid this characteristic may be advantageous particularly by using combined hormones allows collection of eggs from a number of females that have ovulated at different times during the course of a week and fertilization with selected sperm. The control of reproduction by hormonal manipulations and inducing final ovulation are successfully practices in fishes of Caspian Sea (Yousefian *et al.,* 2010; Yousefian and Mousavi, 2008; Mousavi and Yousefian, 2012). Gonadal maturation in teleost fish is primarily regulated by the brain–pituitary–gonadal axis. The GnRH stimulates the synthesis and release of pituitary gonadotropin (Mousavi and Yousefian, 2012; Yousefian and Mousavi, 2011a; Yousefian and Mousavi, 2011b), and GTH stimulates the production of steroid hormones in the gonads (Mylonas *et al.,* 2010; Zohar *et al.,* 2010).

The introduction of GnRH analogs (GnRHa) has proven to be efficient in inducing maturation and spawning in many fish species (Vazirzadeh *et al.,* 2008; Yousefian et al., 2008; Yousefian *et al.,* 2009; Park *et al.,* 2007; Arabaci *et al.,* 2004). Likewise, anti-dopaminergic drugs, domperidone and metoclopramide, have also been found to be highly effective for stimulating the spawning process of fishes mainly in cyprinids and catfishes (Billard *et al.,* 1984; Yousefian *et al.,* 2008). Notwithstanding that

---

Corresponding Author: Seyed Ehsan Mousavi, Department of Fishery, Qaemshahr branch, Islamic Azad University, Qaemshahr, Iran. **E-mail:** se83mousavi@yahoo.com

trout (*Oncorhynchus* and *Salmo* spp.) do undergo final maturation and ovulate during their annual reproductive season, it is still not easy to predict the exact timing of ovulation in individual females, necessitating frequent checking of a broodstock over a period of more than a month (Crim and Glebe,1984). Checking brooders one by one at the time of spawning season in very cold water is an important and difficult part of artificial propagation of salmonid fish species.

There are several methods available for spawning induction: injection of pituitary extracts, human chorionic gonadotropin (HCG), gonadotropin (GtH), gonadotropin-releasing hormone (GnRH) and GnRH agonists. GnRH and its agonists for spawning induction therapies have important advantages over the use of GtH preparations. GnRH can provide a more balanced stimulation of reproductive events and, better integration of the events with other physiological functions, by directly or indirectly affecting release of other hormones necessary for successful final oocyte maturation and spawning (Zohar and Mylonas, 2001; Yousefian and Mousavi, 2011a; Yousefian and Mousavi, 2011b). There are several methods for administration of GnRHa to broodstocks include; injection of hormone in vehicle, the use of GnRHa with dopaminergic drugs, sustained release preparation, of these methods, the best one is the sustained release preparation of GnRHa (Zohar and Mylonas, 2001; Mylonas *et al.,* 2010).

Sustained release preparations of GnRHa replace the multiple GnRHa treatments that are often necessary for a successful response (Zohar and Mylones, 2001). On the other hand, the length of gametogenesis of salmonid species is under gonadotropin control (Mylonas *et al.,* 2010; Arabaci *et al.,* 2004; Vazirzadeh *et al.,* 2008). Thus it could be hypothesized that advancement and synchronization of ovulation would require a more prolonged and controlled stimulation of GtH secretion compared to that required in other species (Zohar and Mylones, 2001).

These delivery systems alleviate the need for multiple treatments, but they are expensive and not readily available worldwide. Therefore, in the present study three different GnRHa and two different anti-dopaminergic drugs were used by double injection protocol. Thereby, the aims of this study were to evaluate; (a) assessing the effect of different kinds of GnRHa (in a same dose, 20 µg per kg body weight) and two different kind of anti-dopaminergic drugs, (b) the effect of GnRHas+DA on advancement and synchronization of ovulation and (c) egg quality in the early spawning strain of Caspian brown trout, *Salmo trutta caspius.*

MATERIAL AND METHODS

The experiment was carried out at the Kalardasht Salmonids Reproduction Center (KSRC), Iran, during the spawning season of Caspian brown trout. Sexually maturing 2-years-old female Caspian brown trout (*S. trutta caspius*) with a mean (±S.D.) weight of 265±18.68 g were randomly selected and transferred to one 6-m3 concrete raceways supplied with running water, approximately 4 weeks prior to the normal spawning (early Dec-Feb) date.

Female/male ratio was set to be 1:2. Broodstocks were maintained under natural water temperature (6.5 ± 0.3 °C) and photoperiod. Fish were acclimated to the holding condition for a week.

2.1. Hormonal Treatment

The treatments included (a) an intraperitoneal injection of a commercial salmon GnRHa/domperidone preparation (Ovaprim, Syndel Laboratories Ltd., India; 20 µg [D-Arg6,Pro9- Net] sGnRHa+ 10 mg domperidone per ml propylene glycol) , (b) an intraperitoneal injection of GnRHa [D-Ala6,Pro9-Net] LHRHa (Ningbo SanSheng Pharmaceutical Co., Ltd, China) in combination with dopamine receptor antagonist, Metoclopramide (Osvah Co., Tehran, Iran), (c) an intraperitoneal injection of GnRHa [D- Trp6-mGnRH] in combination with Metoclopramide, (d) an intraperitoneal injection of GnRHa [D-Arg6,Pro9, Net] sGnRHa in combination with Metoclopramide, (e) a Control group without any treatment). Water temperature was 7.5 °C at the beginning of the experiment. After allowing the fish to acclimatize in a new raceway for one week, they were anesthetized in tricaine methane-sulfonate (MS222, 100 ppm) and female fish (n=50) were randomly divided into five groups (n=10) and injected intraperitoneally with one of five treatments. Fish in group 1 (n=10) received two injections with sGnRHa+domperidone (trade mark Ovaprim) spaced 4 days apart (GnRHa-1). Fish in group 2 (n=10,GnRHa-2), group 3(n=10, GnRHa-3) and group 4 (n=10, GnRHa-4) were injected with GnRHa ([D- Ala6,Pro9-Net] LHRHa, [D-Trp6-mGnRH] and [D-Arg6,Pro9, Net] sGnRHa respectively) diluted with propylene glycol and mixed solutions of GnRHas with Metoclopramide (GnRHa+Met in groups 2,3 and 4) at Day 0 and Day 4. In each injection, fish received half dose of hormone. Control fish (n=10) received only 0.5 ml per kg-1 body

weight of propylene glycol at Day 0 and Day 4. The final concentrations of GnRHa and Met (Dopamine antagonist) were 20 µg GnRHa kg-1 body weight (BW) and 10 mg kg-1 body weight (BW), respectively. Total volume of each injection was 0.5 ml (males were given half the female dose). All treatments were prepared just before the injections.

2.2. Egg Collection and Quality

After treatment, fish were checked for ovulation twice a week, by manually stripping eggs using gentle pressure on the abdominal region. Once ovulation was initiated, fish were checked for ripeness daily. Each ovulated fish was stripped and the eggs were fertilized using sperm from three males. To evaluate egg quality, three samples of about 150 eggs from each female were incubated separately in plastic floating trays. After eyeing, the dead eggs were preserved and cleared in Stockard solution (5:4:6:85 v/v formaldehyde: glacial acetic acid: glycerin: water). Therefore, egg diameters, the fertilization, eyeing (as percentage of fertilized eggs) and hatching rate (as percentage of eyed eggs) were determined (Mylonas *et al.,* 1992).

2.3. Calculations and Statistical Analyses

Data on the mean time to ovulation were analyzed using non-parametric Analysis of Variance (ANOVA, Kruskal–Wallis; SPSS). Egg quality data were analyzed using analysis of variance (ANOVA) after arcsine- transformation of data in case of percentages. Statistical significance was accepted at $P<0.05$. Data is reported as mean±S.D.

RESULTS

3.1. Effects of Gnrha Administration on the Induction and Synchronization of Ovulation in Caspian Brown Trout

Treatment with the GnRHa-1 and GnRHa-2 significantly advanced the spawning date in Caspian brown trout (Figure 1). In all GnRHa treatment groups, ovulation was initiated as early as day 10 after second injection treatments. Ovulation reached 100% on day 14-15 days in the fish receiving the GnRHa-1 and GnRHa-2. In the control group, the cumulative percentage of ovulation increased slowly from day 16 to day 31 reaching 33.33% at the end of the experiment (Day 36). At the end of the experiment (day 36), 50% (GnRHa-3) and 83.33% (GnRHa-4) of fish injected with (GnRHa-DA) had ovulated within 21 and 18 d respectively (Figure 1).

Fish injected with the GnRHa-1, GnRHa-2, GnRHa-3 and GnRHa-4 had a mean time to ovulation of 8.5, 7.2, 15 and 12.5 days, respectively, compared with 24.2 days for control treated fish (Figure 2).

The mean time to ovulation for fish injected with the GnRHa-1 group and GnRHa-2 group were significantly shorter compared with control group and the GnRHa-3 and GnRHa-4 treatment groups ($p < 0.05$).The GnRHa-3 and GnRHa-4 treatment groups advanced significantly the spawning date compared to control group of the Caspian brown trout ($p < 0.05$) (Figure 2).

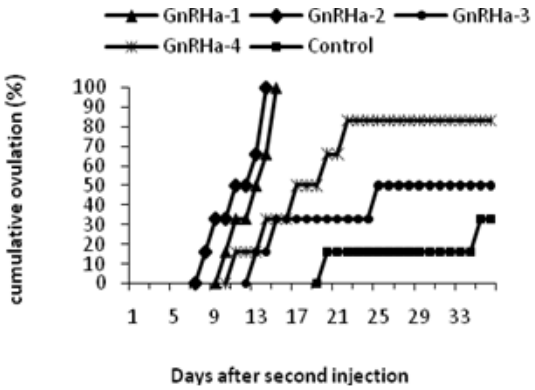

Figure 1: The effect of treatment with GnRHas+Met (group 2,3 and 4) in propylene glycol and GnRHa+Dom (group 1) in propylene glycol on cumulative ovulation of Caspian brown trout.

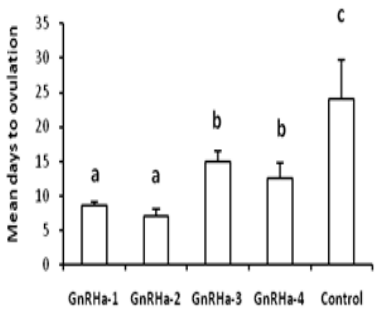

Figure 2: Mean (±S.D.) time to ovulation. Different letter superscripts indicate statistically significant differences among means (ANOVA, $P<0.05$). GnRHa-1: D- Arg6, Pro9- Net plus Domperidone, GnRHa-2: D-Ala6, Pro9-Net plus Metoclopramide, GnRHa-3: D-Trp6-mGnRH plus Metoclopramide, GnRHa-4: D-Arg6, Pro9, Net plus Metoclopramide, Control group: Propylene glycol.

3.2. Effects of Gnrha Treatment on Egg Quality and Post-Spawning Mortality

The fertilization percentage, eyeing percentage, hatching percentage and egg diameters are given Table 1. Fertilization, eyeing, hatching success and egg diameters of the eggs produce by the five treatments were normal and no significant

differences were observed in the parameters mentioned above among groups (p>0.05). In present study, no mortality was observed in the fish given GnRHa+DA treatments intraperitoneally, which were monitored for 2 months after treatment.

Table 1: Mean (±S.D.) of fertilization percentage, eyeing percentage, hatching percentage and egg diameters of eggs produced from Caspian brown trout according to the treatments

Treatment	Fertilization (%)	Eyeing (%)	Hatching (%)	Egg diameters
Control	83±6.8 a	92.8±3.1 a	92±1.8 a	3.8±0.2 a
GnRHa-1	81±7.7 a	92.3±2.2 a	92±0.8 a	3.7±0.1 a
GnRHa-2	78±9.5 a	91.7±2.3 a	91±1.7 a	3.7±0.1 a
GnRHa-3	77±10.4 a	92.4±2.5 a	94±2.2 a	3.7±0.2 a
GnRHa-4	79±8.6 a	92.6±2.6 a	92±2.4 a	3.7±0.1 a

No statistical difference was detected among any of the parameters (ANOVA, P>0.05).

DISCUSSION

In last three decades the use of hormone to induce spawning and synchronizing of ovulation has been of great interest, in order to maximize yield while minimizing the number of pawners (Yousefian *et al.*, 2010). Although Caspian brown trout such as other salmon and trout spawn at specific seasons of the year, it is still not easy to predict with precision the timing of ovulation in individual fish, necessitating frequent checking of captive broodstock over a period of a month or even longer (Crim and Glebe, 1984; Bromage *et al.*, 1992; Arabaci *et al.*, 2004).

Several methods have been used to induce ovulation and spawning in salmonid species. These include salmon gonadotropin preparations (Hunter *et al.*, 1978), gonadotropin and tamoxifen treatment (Donaldson *et al.*, 1981), salmon pituitary extracts (Hunter *et al.*, 1981) Recently, GnRHa+ PIM (Billard *et al.*, 1984; Park *et al.*, 2007) and GnRH agonists have gained favour among commercial fish producers to advance and synchronize of spawning (Mylonas *et al.*, 1992; Breton *et al.*, 1990) The typical effective dose for commercial salmonid species such as rainbow trout and brown trout (*Salmo trutta*) is ranged 10-20 µg kg-1 b.w. of GnRHa and 1-10 mg kg-1 of DA in two injection apart 3-6 days (Billard *et al.*, 1984; Mylonas *et al.*, 1992). Therefore this was used as the median dose in the present study.

Combined injection of GnRHa and Pimozide and the effect of PIM on advancement and synchronization of spawning has been reported in rainbow trout (Billard *et al.*, 1984), Arctic charr (*Salvelinus alpinus*) (Gillet *et al.*, 1996) and Chum salmon (*Oncorhynchus keta*) (Park *et al.*, 2007) that is consistent with its reported role as a dopamine antagonist.

Notwithstanding a controversial role of anti-dopaminergic drugs on ovulation in salmonids, it is evident that dopamine antagonists have a significant impact on influencing the process of final oocyte maturation and ovulation through an indirect pathway, i.e., pituitary LH secretion by GnRHa (Billard *et al.*, 1984; Yaron *et al.*, 2003) For the use and comparing of different GnRHa for the purpose of controlling spawning, GnRH analogues have often proven more effective than native forms because of their resistance to enzymatic degradation (Goren *et al.*, 1990). It was (coincident) with the result we found for Caspian brown trout. Analogues of mammalian and salmon forms, such as those used in the present study have employed, however in comparisons using mammalian (D-Ala6, Pro9, NEt-mammalian) and salmon (D-Ala6, Pro9, NEt-salmon) analogues, no differences in efficacy were found in gilthead seabream (Zohar *et al.*, 1989) or landlocked salmon (Crim *et al.*, 1988b) but the salmon analogue was shown to be more potent in causing gonadotropin release in other species such as the rainbow trout (Crim *et al.*, 1988c).

The failure that observed in group 3 and 4 in compared to group 1 and 2 treatments that reliably induce maturation is probably the result of this short residence time of GnRHa in circulation, which ranges from a few hours to a few days depending on GnRHa, initial dose, fish species and water temperature As stated earlier by Zohar and Mylonas, (2001), FOM may require many days to be completed, and GnRHa must be maintained elevated in the circulation throughout this time in order to induce the necessary elevations in plasma LH.

Also, fish do not begin FOM immediately after GnRHa injection, even though plasma LH or steroid levels may begin to increase within hours after treatment. The results (which part of results)in present study were consisted with other investigations on coho salmon (Fitzpatrick *et al.*, 1984) and brown trout (Mylonas *et al.*, 1992).

In the present study GnRHa+DA were effective in advancing the ovulation time and compressing the ovulation season when using a double injection in all treatment groups. The result showed treatment of GnRHa (group 3 and 4) were significantly lower effect compared with group 1 and 2 in inducing and synchronize ovulation. This failure is probably related to the stimulation of a brief gonadotropin (GtH) secretion, which can be effective only in fish at a very advanced stage of final oocyte maturation as stated earlier by (Breton *et al.*, 1990).

The present study clearly showed that the GnRHa in combination with dopamine antagonist treatment and administration of

GnRHa in double injection protocol were effective, as reported by previous works (Arabaci *et al.,* 2004; Park *et al.,* 2007).

In our study we focused on a practical evaluation of one commercial preparations containing GnRH analogues and three kind of GnRH combined with DA for ovulation stimulation in Caspian brown trout.

Treatments of some GnRH agonists such as those used in present study are effective in inducing ovulation in rainbow trout (Crim *et al.,* 1988a; Crim *et al.,* 1988b; Breton *et al.,* 1990; Pankhurst and Thomas, 1998). The biological activity and/or binding affinity was studied in rainbow trout, landlocked salmon *Salmo solar,* winter flounder *P. americanus* (Crim *et al.,* 1988b) goldfish *Carassius auratus* (Habibi *et al.,* 1989) and African catfish *Claries gariepinus* (De Leeuw *et al.,* 1988).

All treatment by GnRH had positive effect in inducing and synchronizing ovulation that illustrate the potential of this hormone in inducing artificial reproduction. GnRH acts at a higher level of the hypothalamus–pituitary–gonad axis than gonado-tropins. Consequently, GnRH can provide a more balanced stimulation of reproductive events and, presumably, a better integration of these events with other physiological functions, by directly or indirectly affecting the release of other hormones necessary for successful ovulation (Zohar and Mylonas, 2001).

In the other hand, in lower doses, double injections showed better results than single injection; it probably related to rapid degradation and short half-life activity of GnRHa (Zohar *et al.,* 1989), because of cytosolic enzyme activity in pituitary, kidney and liver of the fish (Zohar and Mylonas, 2001). Double injections are preferred for some other fish species such as walleye (*Stizostedion vitreum*) (Pankhurst *et al.,* 1986), brown trout (*Salmo trutta*) (Mylonas *et al.,* 1992).

The overall results of the present study indicate that injection of GnRHa alone or combined with DA accelerates final oocyte maturation and induces ovulation in female Caspian brown trout. The results, however, should be considered preliminary as only three individuals made up that treatment group. Clearly, the dopaminergic inhibition of final maturation and ovulation in Caspian brown trout will require further investigation.

Based on the similar investigations, for the eyed embryos and hatched alevins factors, it is apparent that injections of GnRHa and/or DA into maternal Caspian brown trout did not severely affect egg quality and embryonic development in the present study. In previous investigations, GnRHa alone and/or DA-mixed treatments induced ovulation without significant effects on egg quality in rainbow trout (Arabacı *et al.,* 2004), Arctic charr (Haraldsson *et al.,* 1993) and brown trout (*Salmo trutta*) (Billard *et al.,* 1984).

In contrast, GnRHa+PIM injection in brown trout and GnRHa injection in coho salmon (*O. kisutch*) have also resulted in lower percent fertilization and lower survival at the eyed egg stages (Billard *et al.,* 1984; Fitzpatrick *et al.,* 1984). This discrepancy may be ascribed to early ovulation with incomplete final oocyte maturation due to relatively higher doses of gonadotropin (Mylonas *et al.,* 1992). Inducing ovulation with GnRHa combined with dopamine antagonists did not have any adverse effect on egg viability.

CONCLUSION

In conclusion, GnRHa-DA was effective in advancing the onset of ovulation, synchronizing the ovulation of the treated fish and shortening the reproductive period, relative to the control group. GnRHa-DA treatments did not affect oocyte diameter or egg quality based on the fertilization, eyeing and hatching percentages. This study demonstrated that a combination of GnRHa and DA is an effective and reliable method for induction of ovulation in Caspian brown trout and can be very useful for hatchery and brood fish management in the Caspian brown trout spawning and restocking programs.

ACKNOWLEDGEMENT

Authors would like to thank the staff of the breeding and cultivation center of Kelardasht (KSRC) for providing experimental material.

REFERENCES

Arabaci M, Diler I, Sari M. Induction and synchronization of ovulation in rainbow trout, *Oncorhynchus mykiss*, by administration of emulsified buserelin (GnRHa) and its effects on egg quality. Aquaculture 2004;237:475-484.

Billard R, Reinaud P, Hollebecq MG, Breton B. Advancement and synchronization of spawning in *Salmo gairdneri* and *S. trutta* following administration of LRH-A combined or not with pimozide. Aquaculture 1984;43:57-66.

Bromage N, Jones J, Randall C, Thrush M, Springate J, Duston J, Barker G. Broodstock management, fecundity, egg quality and the timing of egg production in the rainbow trout (*Oncorhynchus mykiss*). Aquaculture 1992;100:141-166.

Breton B, Weil C, Sambroni E, Zohar Y. Effects of acute versus sustained administration of GnRHa on GtH release and ovulation in the rainbow trout, *Oncorhynchus mykiss*. Aquaculture 1990;91:373-383.

Crim LW, Glebe BD. Advancement and synchrony of ovulation in Atlantic salmon with pelleted LHRH analog. Aquaculture 1984;43:47-56.

Crim LW, Arnaud RSt, Lavoie M, Labrie F. A study of LHRH receptors in the pituitary gland of the winter flounder (*Pseudopleuronectes americanus*, Walbaum). Gen Comp Endocrinol 1988a;69:372-377.

Crim LW, Nestor JJ, Wilson CE. Studies of the biological activity of LHRH analogs in the rainbow trout, landlocked salmon, and the winter flounder. Gen Comp Endocrinol 1988b;71:372-382.

Crim LW, Sherwood NM, Wilson CE. Sustained hormone release. Effectiveness of LHRH analog (LHRHa) administration by either single time injection or cholesterol pellet implantation on plasma gonadotropin levels in a bioassay model fish, the juvenile rainbow trout. Aquaculture 1988c;74:87-95.

Donaldson EM, Hunter GA, Dye HM. Induced ovulation in coho salmon (*Oncorhynchus kisutch*): III. Preliminary study on the use of the antiestrogen Tamoxifen. Aquaculture 1981;26:143-154.

De Leeuw R, Vant Veer C, Smit-van Dijk W, Goos HJTh, Van Oordt PGWJ. Binding affinity and biological activity of gonadotropin-releasing analogues in the African catfish, *Clarius gariepinus*. Aquaculture 1988;71:119-131.

Fitzpatrick MS, Suzumoto BK, Schreck CB, Oberbillig D. Luteinizing hormone- releasing hormone analogue induces precocious ovulation in adult coho salmon (*Oncorhynchus kisutch*). Aquaculture 1984;43:67-73.

Gillet C, Breton B, Mikolajczyk T. Effects of GnRHa and pimozide treatments on the timing of ovulation and on egg quality in Arctic charr (*Salvelinus alpinus*) at 5 and 10 °C. Aquat Living Resour 1996;9:257-263.

Goren A, Zohar Y, Fridkin M, Elhanati E, Koch Y. Degradation of gonadotropin releasing hormone in the gilthead seabream, *Sparus aurata*: I. Cleavage of native salmon GnRH, mammalian LHRH and their analogs in the pituitary. Gen Comp Endocrinol 1990;79:291-305.

Hunter GA, Donaldson EM, Stone ET, Dye HM. Induced ovulation of female Chinook salmon (*Oncorhynchus tshawytscha*) at a production hatchery. Aquaculture 1978;15:99-112.

Hunter GA, Donaldson ED, Dye HM. Induced ovulation in coho salmon, *Oncorhynchus kisutch*: I. Further studies on the use of salmon pituitary preparations. Aquaculture 1981;26:117-127.

Habibi RH, Marchant TA, Carol S, Nahorniak CS, Loo HVD, Peter RE, Rivier JE, Vale WW. Functional relationship between receptor binding and biological activity for analogues of mammalian and salmon gonadotropin-releasing hormones in the pituitary of goldfish (*Carassius auratus*). Biol Reprod 1989;40:1152-1161.

Haraldsson H, Sveinsson T, Skulason S. Effects of GnRHa treatments upon the timing of ovulation and upon egg and offspring quality in Arctic charr, *Salvelinus alpinus* L. Aquac Fish Manage 1993;24:145-150.

Mousavi SE, Yousefian M. Effects of exogenous hormones on plasma cortisol, sex steroid hormone and glucose levels in male and female grass carp, *Ctenopharyngodon idellus*, during the spawning induction. African Journal of Biotechnology 2012;11(36):8920-8927.

Mousavi SE, Yousefian M, Masoudnia A, Fahimi S. Effects Of Three Different GnRH Analogues In Combination With Two Different Dopamine Antagonists On Milt Production Of Endangered Non-Spermiating Male Caspian Brown Trout, *Salmo Trutta Caspius*. Advances in Environmental Biology 2011;5(9):2761-2768.

Mylonas CC, Hinshaw JM, Sullivan CV. GnRHa-induced ovulation of brown trout (Salmo trutta) and its effects on egg quality. Aquaculture 1992;106:379-392.

Mylonas CC, Fostier A, Zanuy S. Broodstock management and hormonal manipulations of fish reproduction. Gen Comp Endocrinol 2010;165:516-534.

Pankhurst NW, Thomas PM. Maintenance at elevated temperature delays the steroidogenic and ovulatory responsiveness of rainbow trout, *Oncorhynchus mykiss* to luteinizing hormone releasing hormone analogue. Aquaculture 1998;166:163-177.

Pankhurst NW, Van Der Kraak G, Peter RE. Effects of human chorionic gonadotropin, des-Gly10 (D-Ala6) LHRH-ethylamide and pimozide on oocyte final maturation, ovulation and levels of plasma sex steroids in the walleye (*Stizostedion vitreum*). Fish Physiol Biochem 1986;1:45-54.

Park WD, Lee CH, Lee CS, Kim DJ, Kim JH, Tamaru CS, Sohn YC. Effects of gonadotropin-releasing hormone analog combined with pimozide on plasma sex

steroid hormones, ovulation and egg quality in fresh water-exposed female chum salmon (*Oncorhynchus keta*). Aquaculture 2007;271:488-497.

Vazirzadeh A, Hajimoradloo A, Esmaeili HR, Akhlaghi M. Effects of emulsified versus saline administration of GnRHa on induction of ovulation in rainbow trout, *Oncorhynchus mykiss*. Aquaculture 2008;280:267-269.

Yousefian M, Najafpour SH, Farabi SV, Najafpour GD. Artificial spawning and early development of *Acipenser persicus*. World J Fish and Marine Sci 2010;2(31):258-263.

Yousefian M, Ghanei M, Pourgolam R, Rostami HKH. Gonad Development and Hormonal Induction in Artificial Propagation of Grey Mullet, *Mugil Cephalus* L. Research Journal of Fisheries and Hydrobiology 2009;4(2):35-40.

Yousefian, M, Mosavi H. Spawning of South Caspian Kutum (*Rutilus frisii kutum*) in most migratory river of South Caspian Sea. Asian J Anim Vet Adv 2008;3:437-442.

Yousefian M, Gezel HG, Hedayatifard M. Induction of ovulation in endemic *Chalcarburnus* (sic) *chalcoides*, living in the Caspian Sea, using LRH-Aa combined with metoclopramide. African Journal of Biotechnology 2008;7(22):4199-4201.

Yousefian M, Mousavi SE. A review of the control of reproduction and hormonal manipulations in finfish species. Afr J Agric Res 2011a;6(7):1643-1650.

Yousefian M, Mousavi SE. The mechanism of reproduction and hormonal function in finfish species: A review. Scientific Research and Essays 2011b;6(17):3561-3570.

Yaron Z, Gur G, Melamed P, Rosenfeld H, Elizur A, Levavi-Sivan B. Regulation of fish gonadotropins. Int Rev Cyt 2003;225:131-185.

Zohar Y, Goren A, Tosky M, Pagelson G, Leibovitz D, Koch Y. The bioactivity of gonadotropin-releasing hormones and its regulation in the gilthead seabream, Sparus aurata: in vivo and in vitro studies. Fish Physiol Biochem 1989;7:59-57.

Zohar Y, Mylonas CC. Endocrine manipulations of spawning in cultured fish: from hormones to genes. Aquaculture 2001;197:99-136.

Zohar Y, Muñoz-Cueto JA, Elizur A, Kah O. Neuroendocrinology of reproduction in teleost fish. Gen Comp Endocrinol 2010;165:438-455.