Journal of Reproduction and Infertility 2 (1): 08-12, 2011
ISSN 2079-2166

Effects of Ovaprim (Sgnrha+Dampridon) and Luteotropin Releasing Hormoned-Ala Analog (LRH-A_2) on Artificial Reproduction of Captive Caspian Brown Trout *Salmo trruta caspius* Kessler, 1870

[1]*Amin Farahi,* [1]*Mohammad Sudagar,*
[1]*Seyed Abbas Hoseini and* [2]*Seyed Mohammad Jalil Zorieh Zahra*

[1]Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
[2]Coldwater Fishes Research Center (CFRC),
Lead Center of Network of Aquaculture, Centers in Asia Pacific (NACA)

Abstract: Induced spawning of the Caspian brown trout (*Salmo trutta caspius*) was successfully carried out using ovaprim contains an analogue of salmon gonadotropin releasing hormone and the dopamine antagonis (domperidone) and Luteotropin Releasing Hormoned-Ala Analog (LRH-A_2). Females were administered a single intramuscular injection of the hormone; ovaprim at 0.5 mg kg^{-1} and LRH-A_2 at 2 and 5 μg kg^{-1}. Control group was injected with physiological saline. In LRH-A_2 at 5 μg kg^{-1} were spawned earlier than the other groups (after 3 days). All females in ovaprim and LRH-A_2 groups were ovulated, while females in the control group were not. Hormonal treatments didn't have significant difference in fertilization rate, eyed eggs rate, survival rate of incubation, survival of yolk-sac absorption period and larval deformity. There were significant difference in egg diameter and relative fecundity rate in LRH-A_2 at 5 μg kg^{-1} with other treatments ($p<0.05$) and was higher than them. Also latency period, eyeing period, hatching period and hatching until active feeding period of larvae were shorter in LRH-A_2 at 5 μg kg^{-1}. The results of this study demonstrated that the use of synthetic hormones effectively induce ovulation and spawning in 2-year-old females of Caspian brown trout. These treatments using hypothalamic hormones for Caspian brown trout females could be applied to the development of a method for artificial propagation without a marked deterioration of the egg quality.

Key words: Spawning · Injection · Ovulation · Hormonal treatment · Egg quality

INTRODUCTION

The Caspian brown trout, *Salmo trutta caspius*, belongs to Salmonidae and is an endangered anadromous fish due to overfishing, water pollution and construction of dams and poaching of adults [1, 2]. The Caspian brown trout is one of the economically valuable species in the Caspian Sea. Artificial propagation and production of larvae are the main problems in the early culture of this species for restocking purpose.

Use of exogenous hormones is an effective way to induce maturation of eggs. Furthermore, in the most cultured fishes, hormonal manipulations may be used as management tools to enhance and synchronize egg maturation, spermiation and facilitate hatchery operations [3]. Hormonal preparations applied in fish aquaculture allow improving artificial reproduction techniques both during and outside the spawning season [4-14].

Some species do not reproduce in captivity due to environmental or culture conditions which may cause stress or may not provide the required conditions needed to complete the reproductive process [15].

The gonadotropin-releasing hormone (GnRH) acts at a highest level of the hypothalamus-pituitary-gonad axis and has important advantages more than the use of gonadotropin hormone (GTH). Chemical GnRH synthesis eliminates the risk of the transmission of infectious diseases and also allows the possibility of applying exact doses of GnRH. Another important factor is the high degree of interspecies similarity between GnRH peptides [16] allowing one preparation to be used for more than one fish species. The unsatisfactory potency of natural

Corresponding Author: Amin Farahi, Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Iran, Golestan, Gorgan, Shahid Beheshti Avenue, Postal code: 49138-15739, E-mail: farahi2010@yahoo.com.

GnRH peptides was improved by synthesizing a superactive GnRHa, which is able to induce a significant increase in LH levels even at centuple smaller doses than with the use of natural GnRH forms [17]. Among the GnRHa forms most often used to eliminate reproductive dysfunction in fishes are: [D-Ala^6, Pro^9, NEthylamide]-mGnRH and [D-Tle^6, Pro^9, NEthylamide]-mGnRH, [D-Arg^6, Pro^9, NEthylamide]-sGnRH [18].

The aim of this study was to evaluate effects of ovaprim (SGnRHa+Dampridon) and LRH-A_2 (Luteotropin Releasing Hormoned-Ala Analog) hormones on the breeding quality and comparison efficiency of two stimulators (ovaprim and LRH-A_2) in Caspian brown trout.

MATERIALS AND METHODS

During the reproductive season at Sarshar salmonid fish farm (Tonekabon, Iran), 16 two-year-old females (average weight 683.33±115.47 g) were selected and divided into four groups (4 females in each group). Ten matured males were selected, but were not injected with any hormones. The males had suitable sperm and pool of their sperm was used in this experiment. Females were kept in one area of the raceway and removed by dip net to an anesthetic bath (200 mg clove extract). Females were anaesthetized, weighed and tagged by plastic tags in the dorsal fin. Females were intramuscularly injected with ovaprim (0.5 mg kg^{-1}) and LRH-A2 (2 and 5 μg kg^{-1}). Control group was injected with physiological saline (6.6 m kg^{-1}).

Water temperature was 8 °C which is equivalent to the natural temperature during the recognized spawning period and the temperature for egg incubation was 10 °C. Before obtaining the eggs, sperm of each male was collected, mixed and stored at 4 °C.

After treatment, fish were checked for ripeness third a week, by manually stripping eggs using gentle pressure on the abdominal region. Once ovulation was observed in one individual, the remaining females were checked every day. Each ovulated fish was stripped and the eggs were fertilized using mixed sperm from ten males. Eggs and sperm were gently mixed. Then 500 ml water was added to ensure sperm activation. After 2 min, eggs were rinsed with clean water and left for 30 min. After water-hardening eggs from each female were removed and incubated at 10 °C in mesh baskets placed in Trough. Subsamples of eggs from each female were collected after water-hardening and fertilization (%) was determined on the basis of first cell division, visualized by treatment with a clearing solution (1:1:1 v/v methanol/acetic acid/water) for 2 min. Also relative fecundity, egg diameter, eyed eggs rate, survival rate of incubation, survival of yolk-sac absorption period and larval deformity were determinated.

The data obtained from the trial were subjected to one-way analysis of variance (ANOVA) (using SPSS 16.0 programme) to test for effects of induced treatments. When ANOVA identified significant difference among groups, multiple comparison tests among means were performed using Duncan's new multiple range test. For each comparison, statistically significant differences were determined by setting the aggregate type I error at 5% ($P<0.05$).

RESULTS

According to figure 1 latency period in LRH-A_2 treatment at 5 μg kg^{-1} (3 days) was shorter than the other treatments (11 days). All replications in each treatment had similar latency period.

There was a significant difference among experimental groups in relative fecundity (Table 1). Figure 2 shows that relation between females' body length and their fecundities. This figure indicates that body length didn't affect on fecundity rate, but fecundity rate was influenced by various hormonal treatments.

Eyeing period, hatching period and hatching until active feeding period of larvae were shorter too, in LRH-A_2 treatment at 5 μg kg^{-1} (Figure 3).

Table 1: Reproduction performance in different treatments

Parameters	Control group	Ovaprim 0.5 mg kg^{-1}	LRH-A_2 2 μg kg^{-1}	LRH-A_2 5 μg kg^{-1}
Relative fecundity	-	0.12±0.05^b	0.12±0.03^b	0.22±0.02^a
Fertilization (%)	-	99.05±0.17^a	94.60±4.70^a	97.84±1.81^a
Eyed eggs (%)	-	72.25±11.10^a	56.05±32.22^a	63.87±17.96^a
Hatching (%)	-	95.67±2.17^a	97.35±1.96^a	95.32±2.17^a
Larval survival (%)	-	93.36±0.88^a	87.14±5.24^a	92.11±3.64^a
Deformity (%)	-	2.79±0.26^a	8.27±5.77^a	2.61±0.53^a
Egg diameter (mm)	-	4.98±0.01^b	5.06±0.15^b	5.21±0.06^a

Groups with different alphabetic superscripts differ significantly at $p<0.05$ (ANOVA)

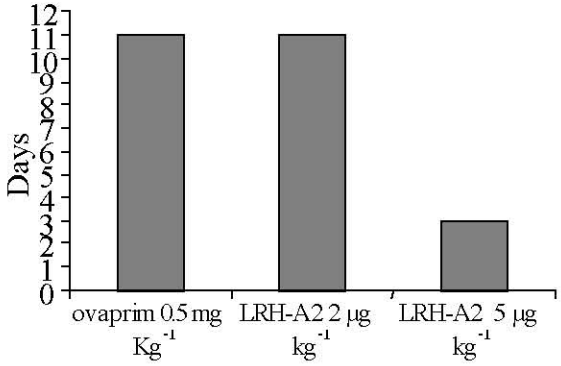

Fig. 1: Latency period in experimental groups

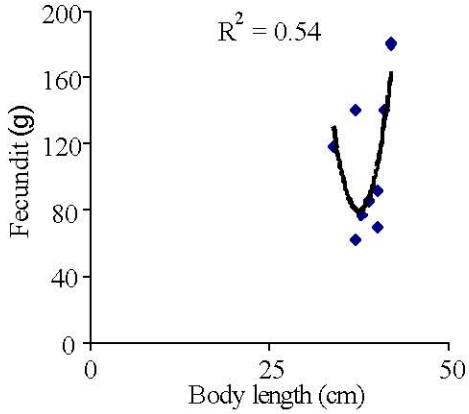

Fig. 2: Relation between body length and females fecundity

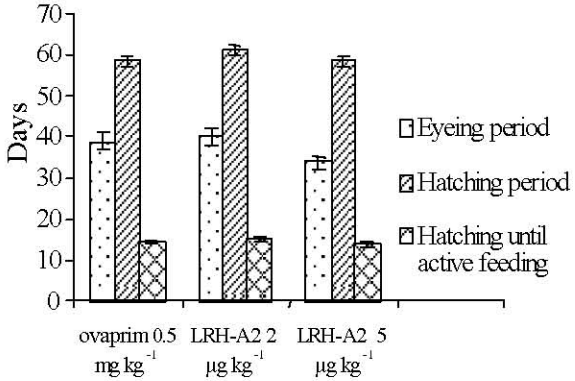

Fig. 3: Different periods in the experimental groups

Ovaprim at 0.5 mg kg^{-1} and LRH-A_2 at 2 and 5 µg kg^{-1} of body weight of females resulted in 100% ovulation of injected females; no females in control group were ovulated. Fertilization rate, eyed eggs rate, survival rate of incubation, survival of yolk-sac absorption period and larval deformity did not differ significantly ($p>0.05$). Egg diameter in LRH-A_2 at 5 µg kg^{-1} showed a significant difference with other groups ($p<0.05$) and was higher than them (Table 1).

DISCUSSION

For the first time in Iran induced spawning of the Caspian brown trout (*Salmo trutta caspius*) was successfully carried out using synthetic hormones. It was a great achievement because of decreasing stocks of this species in Caspian Sea.

Nazari *et al.* [19] with studying the effect of LHRH-A_2 hormone on the induction of final maturation and ovulation of Persian sturgeon found that LHRH-A_2 successfully induced final maturation and ovulation in females. Results of the present study indicate that injection of synthetic hormones accelerates final oocyte maturation and induces ovulation and spawning in Caspian brown trout. These results agree with other findings on multiple species of Haniffa and Sridhar [20], Naeem *et al.* [21], Jamroz *et al.* [22], Metwally and Fouad [23], Adebayo and Papoola [24].

Different latency times after treatment with hormonal preparations were described already earlier [25-27]. In the experiment described two different GnRH analogues that have the same influence and contain different substances. The differences in ovulation time resulting from treatment with different GnRH analogues have been recorded, among others, in case of the carp [28]. In case of the ide a similar situation was also observed by Jamroz *et al.* [22].

Hormonal treatments did not cause any egg quality problem. Fertilization, eyeing and hatching percentages of the eggs were high in all treatment groups. GnRHa treatment induced ovulation without modifying egg quality in rainbow trout [29, 30] and in other salmonid species such as the coho salmon, *Oncorhynchus kisutch* [31]. Other researchers also reported that the use of GnRHa-delivery systems does not compromise the quality of the eggs because there are no differences in fertilization and hatching success between eggs obtained from treated or naturally ovulating fish [32, 33]. Our study is in consistent with successful application of GnRH-a on fertilization rate and incubation survival reported by Goncharov *et al.* [34], Nazari *et al.* [19] and Noori *et al.* [35]. Brzuska [36] reported application of synthetic hormone Des-Gly^{10} [D-Ala^{6}]-LHRH Ethylamide induced a high percentage of deformed larvae while in our study larval deformity was very low and there was no significant difference among experimental groups.

The results of this study clearly showed that LRH-A_2 and ovaprim are effective in stimulating oocyte maturation and ovulation in 2-year-old females Caspian brown trout

in used dosages. Induction of Caspian brown trout to spawning with applicated synthetic hormones appears to affect normal physiological process without causing any abnormalities in reproductive systems and using that can be practically advisable.

REFERENCES

1. Kiabi, B.H., A. Abdoli and M. Naderi, 1999. Status of fish fauna in the south Caspian basin of Iran. Zoo. Mid. E., 18: 57-65.
2. Hatef, A., H. Niksirat, B. Mojazi Amiri, S.M.H. Alavi and M. Karami, 2007. Sperm density, seminal plasma composition and their physiological relationship in the endangered Caspian brown trout (*Salmo trutta caspius*). Aquacult. Res., 38: 1175-1181.
3. Mylonas, C.C., A. Fostier and S. Zanuy, 2010. Broodstock management and hormonal manipulations of fish reproduction. Aquaculture, 165(3): 516-534.
4. Brzuska, E. and J. Adamek, 1999. Artificial spawning of European catfish, *Silurus glanis* L.: stimulation of ovulation using LHRH-a, Ovaprim and carp pituitary extract. Aquacult. Res., 30: 59-64.
5. Kucharczyk, D., R. Kujawar, A. Mamcarz, E. Wyszomirska and D. Ulikowski, 1999. Artificial spawning of ide *Leuciscus idus* under controlled conditions. EJPAU 2(2), 05 (www.ejpau.media.pl).
6. Kucharczyk, D., R. Krol, R. Kujawa, A. Mamcarz, E. Wyszomirska, A. Szczerbowski and M.J. Luczynski, 2000. Out of season spawning of ide (*Leuciscus idus* L.) under controlled conditions. Aquacult., 28: 353-353.
7. Kucharczyk, D., K. Targonska, P. Hliwa, P. Gomulka, M. Kwiatkowski, S. Krejszeff and J. Perkowski, 2008. Reproductive parameters of common carp (*Cyprinus carpio*) spawners during natural season and out-of-season spawning. Reprod. Biol., 8(3): 285-289.
8. Targonska-dietrich, K., T. Zielazny, D. Kucharczyk, A. Mamcarz and R. Kujawa, 2004. Out-of-season spawning of cultured ide (*Leuciscus idus* L.) under controlled conditions. EJPAU., 7(2): 02 (www.ejpau.media.pl).
9. Ulikowski, D., 2004. European catfish (*Silurus glanis* L.) reproduction outside of the spawning season. Arch. Pol. Fish, 12(2): 121-131.
10. Targonska, K., D. Kucharczyk, A. Krasucka and A. Mamcarz, 2005. Artificial reproduction of minnow (*Phoxinus phoxinus*) in captivity. Aquaculture Europe 2005 Conference "Optimizing the Future", Trondheim, Norway, pp: 5-8.
11. Targonska, K., D. Kucharczyk, A. Mamcarz, J. Glogovski, S. Krejezeff, M. Prusinska and K. Kupren, 2008. Influence of individual variability in the percentage of motile spermatozoa and motility time on the survival of embryos of chosen fish species. Pol. J. Natur. Sci., 23(1): 178-187.
12. Cejko, B.I., J. Glogowski, R.K. Kowalski, D. Kucharczyk and K. Targonska, 2008a. Description of pikeperch, *Sander Lucioperca* L., semen obtained from males held under different rearing conditions. Arch. Pol. Fish, 16(1): 93-100.
13. Cejko, B.I., D. Kucharczyk, K. Targonska, D. Kubiak, B. Starosiek and J. Glogowski, 2008b. Quality parameters and selected biochemical markers of asp, *Aspius aspius* (L.), semen obtained after hormonal stimulation with Ovaprim or Ovopel. Arch. Pol. Fish, 16(2): 179-188.
14. Cejko, B.I., R.K. Kowalski, D. Kucharczyk, K. Targonska, S. Krejszeff and D. Zarski and J. Glogowski, 2009. Influence of the length of time after hormonal stimulation on selected parameters of milt of ide *Leuciscus idus* L. Aquacult Res., 41(6): 804-813.
15. Mylonas, C.C. and Y. Zohar, 2001. Use of GnRHa-delivery systems for the control of reproduction in fish. Rev. Fish Biol., 10: 463-491.
16. Chen, C.C. and R.D. Fernald, 2008. GnRH and GnRH receptors: distribution, function and evolution. J. Fish Biol., 73: 1099-1120.
17. Kouril, J., A. Svoboda, J. Hamackova, P. Kalab, J. Kolarova, A. Lepicova, M. Sedova, L. Savina, P.M. Rendon, Z. Svobodova, T. Barth and B. Vykusova, 2007. Repeated administration of different hormonal preparations for artificial propagation and their effects on reproduction, survival and blood biochemistry profiles of female tench (*Tinca tinca* L.). Czech J. Anim. Sci., 52: 183-188.
18. Podhorec, P. and J. Kouril, 2009. Induction of final oocyte maturation in Cyprinidae fish by hypothalamic factors: a review. Vet. Med., 54(3): 97-110.

19. Nazari, R.M., M. Modanloo, M.R. Ghomi and M.R. Ovissipor, 2009. Application of synthetic hormone LHRH-A_2 on the artificial propagation of Persian sturgeon *Acipenser persicus*. Aquacult. Int., 18(5): 837-841.
20. Haniffa, M.A. and S. Sridhar, 2002. Induced spawning of spotted murrel *Channa punctatus* and catfish *Heteropneustes fossilis* using human chrionic gonadotropin and synthetic hormone (ovaprim). Vete. Arch., 72(1): 51-56.
21. Naeem, M., A. Salam and A. Jafar, 2005. Induced spawning of major carp *Catla catla* by a single intramuscular injection of ovaprim-C and Fecundity at fish hatchery Islamabad, Pakistan. J. Biol. Sci., 5(6): 776-780.
22. Jamroz, M., D. Kucharczyk, A.H. Batzowska, S. Krejszeff, R. Kujawa, K. Kupren, M. Kwiatkowski, K. Targonska, D. Zarski, B.I. Cejko and J. Glogowski, 2008. Camparing the effectiveness of ovapel, ovaprim and LHRH analogue used in the controlled reproduction of IDE, *Leuciscus idus* (L.). Polish fisheries, 16: 363-370.
23. Metwally, M.A.A. and I.M. Fouad, 2008. Some biochemical changes associated with injection of grass carp *Ctenopharyngodon idella* with ovaprim and pregnyl for induction of artificial spawning. Glob. Vete., 2(6): 320-326.
24. Adebayo, O.T. and O.M. Papoola, 2008. Comparative evaluation of efficacy and cost of synthetic and non-synthetic hormones for artificial breeding of African catfish *Clarias gariepinus*. J. Fish. and Aqua. Sci., 3(1): 66-71.
25. Yaron, Z., 1995. Endocrine control of gametogenesis and spawning induction in the carp. Aquacult. Res., 129: 49-73.
26. Brzuska, E., 2000. Artificial spawning of carp *Cyprinus carpio* L.: differences between the effects on reproduction in females of Polish and Hungarian provenance treated with carp pituitary and (D-Ala6) GnRH ProNHEt (Kobarelin). Aquacult. Res., 31: 457-465.
27. Brzuska, E., 2005. Artificial spawning of carp (*Cyprinus carpio* L.): differences between females of Polish strain 6 and Hungarian strain W treated with carp pituitary homogenate, Ovopel or Dagin. Aquacult. Res., 36: 1015-1025.
28. Kupren, K., 2008. Influence of individual variability in the percentage of motile spermatozoa and motility time on the survival of embryos of chosen fish species. Pol. J. Natur. Sci., 23(1): 178-187.
29. Pankhurst, N.W. and P.M. Thomas, 1998. Maintenance at elevated temperature delays the steroidogenic and ovulatory responsiveness of rainbow trout *Oncorhynchus mykiss* to luteinizing hormone releasing hormone analogue. Aquacult., 166: 163-177.
30. Arabaci, M., I. Diler and M. Sari, 2004. Induction and synchronisation of ovulation in rainbow trout, Oncorhynchus mykiss, by administration of emulsified buserelin (GnRHa) and its effects on egg quality. Aquaculture, 237: 475-484.
31. Fitzpatrick, M.S., B.K. Suzumoto, C.B. Schreck and D. Oberbillig, 1984. Luteinizing hormone-releasing hormone analogue induces precocious ovulation in adult coho salmon (*Oncorhynchus kisutch*). Aquacult., 43: 67-73.
32. Zohar, Y., G. Pagelson, Y. Gothilf, W.W. Dickhoff, P. Swanson, S. Duguay, W. Gombotz, J. Kost and R. Langer, 1990. Controlled release of gonadotropin releasing hormones for the manipulation of spawning in farmed fish. Control. Release Bioact. Mater, 17: 51-52.
33. Goren, A., H. Gustafson and D. Doering, 1995. Field trials demonstrate the efficacy and commercial benefit of a GnRHa implant to control ovulation and spermiation in salmonids. In: F.W. Goetz, P. Thomas, (Eds.), Reproductive Physiology of Fish, 1995. Fish Symposium 95, University of Texas, Austin, Texas, pp: 99-101.
34. Goncharov, B.F., L.V. Igumnova, I.S. Polupan and E.A. Savieleva, 1991. Inducing oocyte maturation, ovulation and spermation in sturgeons. 1 ISS. CEMAGREF publication.
35. Noori, A., B. Mojazi Amiri, A. Mirvaghefi and D. Baker, 2010. LHRHa-induced ovulation of the endangered-Caspian brown trout (Salmo trutta caspius) and its effect on egg quality and two sex steroids: testosterone and 17α-hydroxyprogestrone. Aquacult., 41(6): 871-877.
36. Brzuska, E., 2003. Artificial propagation of African catfish (*Clarias gariepinus*): differences between reproduction effects after stimulation of ovulation with carp pituitary homogenate or GnRH-a and dopaminergic inhibitor. Czech J. Anim. Sci., 48(5): 181-190.