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EFFECT OF DIFFERENT DOSES OF SYNTHETIC HORMONE LHRH-A_2 ON SERUM SEX HORMONES, OVULATION PERCENT AND EGG HATCHING RATES OF PERSIAN STURGEON *Acipenser persicus*

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ABSTRACT

Acipenser persicus is one of the economically valuable fishes in the Caspian Sea. Due to the economical and cultural importance of sturgeons in the world, understanding of sturgeon reproduction is necessary for successful management of their populations in aquaculture and nature. To improve the culture methods and to facilitate captive breeding programs to support restocking, it is necessary to understand the changes in steroid hormones during artificial reproduction. The administration of sturgeon luteinizing hormone - releasing hormone analogue (LHRH-A_2) induces changes in serum sex steroid levels. To study the effects of different doses of LHRH-A_2 hormone on induction of final maturation and ovulation of Persian sturgeon, 18 maturating females and 18 maturating males were used. All brooders were injected with LHRH-A_2 in dosages of 4, 6 and 8 μg kg^{-1}. In this study, LHRH-A_2 successfully induced final maturation and ovulation in females, and the highest percentage of ovulated females was observed in the groups treated with dose of 4 μg kg^{-1} LHRH-A_2. There were significant differences in serum cortisol (C), testosterone (T) and Gonadotropin hormone (GTHII) concentrations at tested dosages. In hormonal treatment groups, the highest hatching rate was observed in the groups receiving 4 μg kg^{-1} LHRH-A_2. Finally, the LHRH-A_2 in dose of 4 μg kg^{-1} is effective in stimulating oocyte maturation and ovulation in Persian sturgeon.

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INTRODUCTION

Persian sturgeon *Acipenser persicus* is an anadromous fish belonging to the genus *Acipenser* and to the family Acipenseridae (Holcik, 1989). This species migrates to the spawning sites in Sefid Rood at the end of April and in May, and also in autumn between September and October (Taghavi Motlagh, 1996). Sturgeon stocks are dramatically decreasing (Chebanov and Billard, 2001). This decline resulted from overfishing, poaching and environmental degradation such as accumulation of pollutants in sediments, damming of rivers and restricting waterflow, which become unfavorable to migration and reproduction (Dettlaff et al., 1993). The artificial propagation and the rearing of sturgeons are very important ways used for restocking and conservation of sturgeon populations (Kohneshahri and Azari Takami, 1974). Control of reproduction in fish requires the manipulation of gametes. For development of efficient artificial insemination techniques, basic knowledge on physiology of reproduction is necessary (Billard and Jensen, 1996).

Adult fish, especially the females, are generally unable to produce their gametes naturally under hatchery conditions. The most cost- and time-effective way to obtain ovulated eggs and sperm is stimulating the fish with hormones. Nowadays, pituitary injections are used for induction of ovulation in many hatcheries, but they are replaced by synthetic analogs of the luteinizing hormone releasing hormone (LHRH-A_2) (Chebanov and Billard, 2001) as gonadotropin releasing hormone agonists (GnRHa) which stimulate secretion of endogenous gonadotropin hormone (GTH) (Zohar and Mylonas, 2001). The GnRH acts at the highest level of the hypothalamus–pituitary–gonad axis and has important advantages compared to using GTH. In this approach, different LHRH forms and their analogues are used with a dopamine receptor antagonist (DA) which reduces the dopamine inhibitory action and stimulates GTH releasing (Lin et al., 1986; 1988; Peter et al., 1988). Among the GnRHa forms most often used to eliminate reproductive dysfunction in fishes are: [D-Ala^6, Pro^9, and NEthylamide]-mGnRH, [D-Tle^6, Pro^9, NEthylamide]-mGnRH, [D-Arg^6, Pro^9, NEthylamide]-sGnRH (Podhorec and Kouril 2009). The objective of this study was to determine optimum dose of LHRH-A_2 hormone (D-Ala6 GnRH Pro9-NEt) for the breeding quality in Persian sturgeon.

MATERIALS AND METHODS

During the season of artificial reproduction, 18 Persian sturgeon females (total length = 131.91 ± 12.25 cm: total weight = 12.43 ± 1.93 kg) and males (total length = 121.91 ± 14.08 cm: total weight = 10.6 ± 3.23 kg) were captured in the southern part of the Caspian Sea and transported to Shahid Beheshti Artificial Sturgeon Propagation and Rearing Center (SAPRC), Rasht, Iran. After delivery to SAPRC, the females were kept in the pond (1m×4m×8m; water used had the following conditions: about 1.5 m3/min in pond entrance; water temperature: 16-18°C; dissolved oxygen: 8-8.3 mg/l and pH: 7.3-7.5). First of all, egg sampling was conducted from all females during their expected month of spawning by a probe. Sampled eggs are analyzed for Polarization Index (PI) and also tested for their capacity to undergo Germinal Vesicle Breakdown (GVBD). To this end, the egg samples are boiled (3-5 hours), chilled on wet ice, stored in 10% buffered formalin, bisected and examined for GVBD. The females with Polarization Index (PI: the ratio of the distance of the germinal vesicle from the animal pole over the animal-vegetal oocyte diameter × 100) of 6-9 were selected for induction of spawning (Azari Takami, 1999). All selected brooders were intramuscularly injected with LHRH-A_2 in dosages of 4, 6 and 8 μg kg^{-1} of body weight. Female ripening was checked and observed every 1 h after the injection. Half an hour before the expected time of ovulation, sperm of each male was separately collected with 50-ml polyethylene syringes, and its quality was assessed by sperm motility test and stored in the refrigerator at 4ºC.

Blood samples were taken from the caudal vein of females using heparinized syringe (with maximum degree of 5 ml) at the time of ovulation. Then, the blood samples were centrifuged (3000 rpm for 10 min) (Labofuge200, Heraeus Company, Germany) to separate the serum and then it was stored at − 20°C until hormonal analysis. The glucose concentration was measured spectrophotometrically (Jenway, England) with a diagnostic kit (Pars Azmoon Co, Tehran, Iran). Steroids levels (Cortisol, Testosterone) and GTHII concentrations (ng/mL) were measured by Enzyme-Linked Immunosorbent Assay (ELISA) according to Barannikova et al. (2002, 2004) and Semenkova et al. (2002). Assay kits were purchased from Immunotech Company, France. Each commercial assay kit contained two necessary reagents, i.e. antibody and tracer (labeled antigen). Aliquots containing antibody and tracer were transferred to assay tubes (polystyrene tube). Tracer and antibody were added in a further 100 μl of assay buffer and the tubes were left to incubate overnight at 4 C. The content of polystyrene tubes (antibody, tracer and serum sample) was discharged completely and then polystyrene tubes were applied to a Gamma counter (LKB 182 Compugamma CS, LKB Wallac, Finland) for the measurement of absorption spectrum and subsequently calculation of hormone concentration by using related standard curve. Female breeders were transferred to hatchery hall and their gills were cut to take their blood. Then, the fish were wiped with dry towels and eggs put into plastic dishes. The coelomic fluid was removed by pouring the eggs onto a screen suspended over a beaker and eggs immediately used for propagation. The eggs were then inseminated with semen after eliminating adhesiveness (Dettlaff et al., 1993; Nazari et al., 2010). Then the eggs were placed into Yoshchenko incubators in running freshwater system at 17–19ºC. The variables including percentage of ovulation, fertilization rate, hatching rate and number of eggs per gram were determined. For calculation of fertilizing rate, 4 h after fertilization, 200 eggs were randomly preserved in 10% formalin. Among egg samples, only eggs containing 4 cells were considered as fertilized eggs (Billard, 1986). Hatching occurred between 5-6 days at water temperature of 20 - 24.5°C. Also, number of larvae per gram and malformed larvae were measured after 5-day incubation period in incubators, and dead eggs were counted daily.

The SPSS software was used for data analysis. All data had normal distribution according to Kolmogorov Smirnov test. One-way analysis of variance (ANOVA) was employed to compare the means. When significant F-ratios were calculated by ANOVA, the Duncan test was applied to identify which means were different. For the comparison of the means before and after ovulation, the independent samples t-test was used.

RESULTS

Application of LHRH-A_2 in dosages of 4 μg kg^{-1} of body

weight of females resulted in ovulation of 100% injected fishes and in dosage of 6 and 8 μg kg^{-1} caused the ovulation of 68% fishes (Fig. 1A). Using LHRH-A_2 in dosages of 4, 6 and 8 μg kg^{-1} caused significant differences in Testosterone (Fig. 1 B and C), Cortisol concentrations (Fig. 1 B and C) and the mean plasma concentration of GTH-II (Fig.1D) during ovulation, but glucose concentration did not show significant difference (Fig.1E).

According to Table 1, hatching rate showed significant difference (P<0.05) between the female broods injected with LHRH-A_2 in all dosages, but the results of fertilization rate and number of eggs per gram did not show significant difference. The rate of 100% spermiation was exhibited by all male breeders.

DISCUSSION

The range of effective doses of GnRHa considerably vary between 5 and 100 μg kg^{-1} in different fish species (Kucharczyk et al., 2005; Heyrati et al., 2007; Nazari et al., 2010). According to our results, application of LHRH-A_2 in dose of 4 μg kg^{-1} of body weight resulted in 100% ovulation in injected females. Induction of female Persian sturgeon in dosages of 3.5, 7, 8 μg kg^{-1} of body weight resulted in 100% ovulation in injected females and only in dosage of 10 μg kg^{-1} caused 80% ovulation (Nazari et al., 2010). Also, Doroshov et al. (1997) induced adult females of *Acipenser transmontanus* with dose of 10 μg kg^{-1} of GnRHa followed by intramuscular injection of 4.5 mg kg^{-1} carp pituitary material after 12 hours. Additionally, treatments of some GnRH agonists such as [D-A1a^6, Pro9-NEt -mGnRHa, [D-Arg^6,Pro^9-NEt]-sGnRHa, D-Trp6-mGnRHa, des-Gly10[D-Ala^6]-GnRHa were effective in inducing ovulation in many teleost fish (Pankhurst and Thomas, 1998; Breton et al., 1990).

As sturgeon females progresses from the vitellogenic period to ovulation, the contents of sex steroids and vitellogenin in sturgeon blood and egg follicles fluctuate (Moberg et al., 1991; Doroshov et al., 1997; Patino et al., 2001; Goncharov, 2002). Furthermore, androgens such as T and 11-ketotestosterone (11-KT) may also have a role during final oocyte maturation in sturgeon (Semenkova et al., 2006). Additionally, biochemical components in sturgeon plasma including sex steroids can be used to predict sexual maturity in female fish (Lin et al., 1996). Results of this study were in agreement with the study conducted by Webb et al. (2000) and Semenkova et al. (2006). They reported the elevation of T during final oocyte maturation in sturgeon.

Ovulation in sturgeon is under control of gonadotropin-I (GTH-I) and gonadotropin-II (GTH-II). In this study, GTH-II decreased gradually with increasing dosage of LHRH-A_2. This result was in agreement with the study conducted by Moberg et al. (1995) as they reported the increase of pituitary GTH-II just prior to spawning in sturgeon females. Just during this period, the GTH-II becomes predominant gonadotropin in the pituitary. The efficiency of hormonal

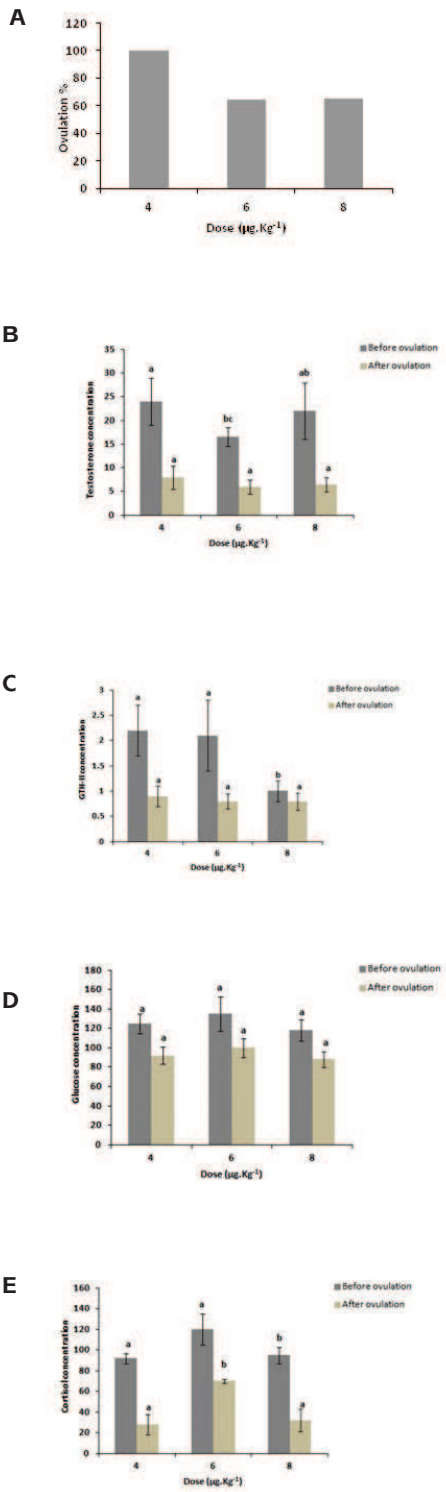

Fig 1. Effect of different doses of LHRH-A2 (A) on percentage of ovulation in female Persian sturgeon and the mean plasma concentration of testosterone (B), GTH-II (C), glucose (D) and cortisol (E) in female Persian sturgeon induced by LHRH-A2

Table 1. Comparison effect of different dosage of LHRH-A_2 on reproduction characteristics

Parameters	Treatments		
	4 µg kg^{-1}	6 µg kg^{-1}	8 µg kg^{-1}
Fertilization rate (%)	89 ± 1.90	82 ± 2.15	85 ± 1.07
Hatching rate (%)	79 ± 3.4 [a]	67 ± 3.79 [b]	74 ± 1.78 [ab]
number of eggs per gram	52 ± 2.40	57 ± 1.99	53 ± 0.98

*The values with different letters are significantly different ($P < 0.05$)

treatments can be evaluated by examining spawning success after hormonal treatments. According to Table 1, the results of fertilizing rate and number of eggs per gram did not show a significant difference ($P>0.05$) between the female brooders injected with LHRH-A2 in all dosages. These results are consistent with successful application of GnRH-a, reported by Goncharov et al. (1991) in *Acipenser stellatus*, *Acipenser gueldenstaedti*, *Huso huso* and *Acipenser ruthenus,* on fertilization rate. In African catfish, higher egg numbers were obtained when GnRH-a was used to synchronize spawning (Brzuska, 2003). Other researchers found that application of GnRHa does not compromise the quality of eggs because there are no differences in fertilization and hatching success between eggs obtained from treated or naturally ovulating fish (Mylonas et al., 1992). Also, some studies showed that the quantity of eggs was higher after the application of synthetic ovulation stimulators (Brzuska, 2002). But in another study, use of 50 µg/kg and 20 µg/kg des-Gly10 (D-Ala^6)-Ethylamide did not affect the number of obtained eggs.

The result of this study clearly demonstrated that LHRH-A_2 in dosage of 4 µg kg^{-1} of body weight is effective in stimulating maturation and ovulation in Persian sturgeon oocytes. Fertilization success also shows better results when LHRH-A_2 was used in dosage of 4 µg kg^{-1} of body weight.

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Sažetak

UTJECAJ RAZLIČITIH DOZA SINTETIČKOG HORMONA LHRH-A_2 NA RAZINE SPOLNIH HORMONA U SERUMU, POSTOTAK USPJEŠNOSTI OVULACIJE I STOPE VALJENJA JAJA PERZIJSKE JESETRE, *Acipenser persicus*

Acipenser persicus (Acipenseridae) jedna je od ekonomski važnijih vrsta riba u Kaspijskom moru. Poznavanje reproduktivnih karakteristika jesetre je važno zbog uspješnog upravljanja njihovim populacijama u akvakulturi te njihove ekonomske i kulturne važnosti u svijetu. Razumijevanje razina steroidnih hormona i njihovih promjena u serumu tijekom umjetne oplodnje pomaže poboljšanju kvalitete nasadnih metoda te olakšava izvedbu programa razmnožavanja u zatočeništvu u svrhu potpore poribljavanja. Primjenom sintetičkog luteinizirajućeg hormona jesetre - oslobađajućeg analognog hormona (LHRH-A_2) - induciraju se promjene u razinama spolnih steroida u serumu. Za proučavanje učinka različitih doza hormona LHRH-A_2 na izazivanje konačnog sazrijevanja i ovulacije Perzijske jesetre, korišteno je 18 odraslih ženki i 18 odraslih mužjaka. Sve muške i ženske spolno zrele jedinke su inducirane s LHRH-A_2 u dozama od 4, 6 i 8 µg kg^{-1}.

U ovoj studiji primjenom LHRH-A_2 uspješno je inducirano sazrijevanje gonada i ovulacije u ženki, dok je najveći postotak ovuliranih ženki uočen u skupini tretiranoj dozom od 4 µg kg^{-1} LHRH-A_2. Postoje značajne razlike u koncentracijama kortizola (C), testosterona (T) i gonadotropina (GTHII) u serumu ispitivanih doza hormona. Najveći postotak valjenja uočen je u skupinama riba koje su primale 4 µg kg^{-1} LHRH-A_2. U dozi od 4 µg kg^{-1}, LHRH-A_2 je učinkovit pri poticanju sazrijevanja oocita te ovulacije Perzijske jesetre.

Ključne riječi: umjetno osjemenjivanje, ovulacija, LHRH-A_2, *Acipenser persicus*

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