Evaluation of LHRH-a acute release implantation on final maturation and spawning in not-fully matured broodstocks of Persian sturgeon (*Acipenser persicus* Borodin, 1897)

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Abstract

The Persian sturgeon (*Acipenser persicus*) is considered as an endemic sturgeon of the south part of the Caspian Sea and provides the highest Iranian caviar production. Due to overfishing, degradation of the rivers conditions of the natural reproductive habitats, the fish stocks is decreasing. The immature breeders do not response to hormonal therapy at the sturgeon hatcheries as most having PI (above 10) and large numbers of breeders caught and transported to the hatcheries were unable to reproduce. This study was attempted to find the effect of LHRHa implantation on oocytes maturation and spawning of Persian sturgeon. Broodstocks were caught from the southeast region of the Caspian Sea. The selected female broodstocks (PI>10) ranged in size from 24.0 to 37.5 kg were implanted with LHRHa cholesterol pellets at concentrations of 0 µg/kg (control), and treatments of 10, 15 and 20 µg/ kg (in three replicates. The results from this study indicated that females treated with LHRHa hormone implantation at 10, 15, 20 µg per kg body weight reached final maturation. These results were observed for all fish from treatment numbers 2 (15 µg/ kg) and 3 (20 µg/kg), however only one fish reached final maturation in treatment 1(10 µg/ kg). The current implantation of LHRHa was able to enhance breeders with PI above 10 to final maturation which under normal condition at sturgeon's hatcheries this is not possible. The results suggested that final maturation can be achieved that lead to high fertilization (78.33% ±13.87, 68.33% ±4.16 in treatment 2 and 3, respectively) and hatching rates (85.3% ±9.07, 68.33% ±7.64 in treatment 2 and 3, respectively) and also total larvae production. Thus, the information from this study is very useful for artificial propagation of not-fully-matured females of Persian sturgeon at Sturgeon hatcheries especially in Iran.

Keywords: *Acipenser persicus*, Broodstocks, LHRHa implantation, Artificial propagation, Fertilization, Hatching rates

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Introduction

Its full scientific name is *Acipenser persicus* (Borodin, 1897). This species is Euryhaline and the majority occurs in typical marine Caspian waters. The name *persicus* was obviously chosen because of the frequent occurrence of this particular species in the southern part of the sea, and along the shores of Iran Persia (Holcik, 1989). In the recent years, the developed researches were made on spawning and maturation pattern in marine fishes (Kaymaram et al., 2010; Salamat et al., 2010) in local area Persian sturgeons form the largest proportion of the total Persian sturgeon commercial catch (Moghim et al., 2005). The Persian sturgeon breeding and fingerling production season in these hatcheries in Iran begins in April and it lasts until July annually. Due to the fact that there are a few number of broodstocks migrating to Iranian rivers to spawn, the artificial propagation process is dependent upon the broodstocks caught in the Caspian Sea.

The Persian sturgeon is an endangered species that possess an elongated and bulky body. The stocks are recovered mainly by artificial propagation and annually more than 18 million fingerlings (weighing around 3-5g) are released to the adjacent rivers of the Caspian Sea from different Iranian hatcheries. It is important to highlight that an artificial propagation of sturgeons for larval production began in 1922 in Iran, with the first sturgeon hatchery constructed in 1971 in the Guilan province (Abdolhay and Tahori, 2005). Currently, there are a total of six active sturgeon hatcheries in Iran, and these are located in the southern region of the Caspian Sea. The total pond area was estimated to be 538 ha (Moghim et al., 1996). Although most of the selected female broodstocks have reached the advanced stage of gonadal maturity, as determined by sampling oocytes and calculating polarization Index and the Germinal vesicle break down (GVBD) response in the oocyte maturation assay (stage IV), many of them still do not respond properly to hormonal treatment, and subsequently do not reach their final maturation.

For the Persian sturgeons, the female broodstocks having Polarization Index (PI) 6-8 are generally selected for spawning induction. Many of the selected broodstocks for artificial reproduction to natural stocks restoration possess PI higher than 8 (PI 10 - 15). The main reason for this phenomenon may be due to the catching of the broodstocks from the Caspian Sea before spawning, while these broodstocks may not be ready physiologically to migrate, and several concepts have also been proposed in relation to their endocrine situation. Meanwhile, it has also been assumed that one or more components of the Brain–Pituitary–Gonad (B-P-G) axis may not have been fully functional (Lee et al., 1986). Another assumption is the normal surge of Gonadotrophin Hormone (GtH), which stimulates the final maturation and ovulation, which does not take place because of environmental situation. On the basis of this information, and also the administration of hormonal implantation

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Luteinising hormone releasing hormone (LHRH) to improve gonadal maturation of some other fish the usage of hormonal treatment to stimulate the H-P-G axis of these broodstocks has been recommended. It is important to note that most studies on chronic and continuous administration of drugs or hormones are carried out on human. However, there are some good studies on teleosts in relation to hormone implantation to improve gonadal maturation. The technique used in making chronic–release LHRHa and 17 –α-Methyltestosterone pellets for intramuscular implantation has been established in fish.

Induction of ovulation by gonadotropin-releasing hormone analogue LHRHa is a common technique used to control reproduction of commercial fish (Zohar, 1996). Among the different types of LHRHa, wD-Ala6–Pro9-Netx-luteinising hormone releasing hormone, i.e. an analogue of mammalian luteinising hormone releasing hormone, is not only efficient when injected into fish, but it is also relatively cheap. It has successfully been used to induce spawning in many species, including salmonids (Mylonas et al., 1995), gilthead seabream (Zohar *et al*., 1989), winter flounder (Harmin and Crim, 1992), striped bass (*Mylonas* et al., 1995), and yellowtail flounder (Larsson et al., 1997). A single injection of LHRHa was found to be effective on more matured females. Two injections were necessary for less advanced fish. Depending on our knowledge, there have been no published studies on the effects of LHRHa implants on female sturgeons. Implants can be designed to give a low and sustained release of LHRHa over a period of several weeks' (Zohar, 1996), and have a major advantage over injections in that the fish only need to be treated and handled once.

The main objectives of the study was to investigate the effect of different dosages of LHRHa implantation in accelerating final oocyte maturation , ovulation and also some artificial propagation indices in not fully matured broodstock of *A. persicus*.

Materials and methods

Study area

The field activities were carried out at Shahid Marjani sturgeon hatchery which is located in the Golestan province in 2008. Meanwhile, laboratory activities were conducted at Golestan Inland Waters Aquatics Stocks Research centre in the Gorgan city from 2008 to 2009.

Breeders' source

All gravid females as well as ripe male sturgeons were captured at south coastal waters of the Caspian Sea. At present study, wild Persian sturgeon broodstocks after catching were transported in a truck fitted with a large holding tank and oxygen supply to the sturgeon hatchery and maintained in an outdoor circular fresh flow through freshwater system.

Treatments

Twelve broodstocks of Persian sturgeons with PI more than 10 (ranged from 10.5 to 15.4) were selected. Four treatments were given to the broodstocks to be implanted intramuscularly with LHRH-A cholesterol pellet in the concentrations of 0 ug/kg (control), 10 ug/kg (treatment 1), 15 ug/kg (treatment 2) and 20 ug/kg (treatment 3).

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Three fish were considered for each treatments and control.The LHRH-A cholesterol pellets were prepared according to Lee et al. (1986).

Fertilization and De-adhesion of Eggs

Artificial propagation and eggs incubation were carried out according to Dettlaff et al. (1993). The time required for ovulated eggs collection (by abdominal opening) is dependent on the species of fish, size of the female, fecundity and also the skill of the person who collects sturgeon eggs. The eggs should be removed by opening the female abdomen. Fertilization should take place within 10-20 minutes after the collection of eggs. The ova in each bowl were mixed with pooled milt taken from 3-4 males at a rate of 10 ml milt per kg of the ovulated eggs. The oocytes and milt mixture was gently mixed for about 5 minutes or until the eggs started to stick to the sides of bowls. Using a large feather by hand, the oocytes and milt were mixed. After fertilization was completed, the water-milt solution was decanted off. As sturgeon eggs become sticky after fertilization, it is necessary to neutralize the sticky jelly coat to prevent adhesion to each other during incubation stickiness neutralized by one hour treatment in a continuously moving clay water suspension (Dettlaff et al., 1993).

Incubation

After egg de-adhesion, the number of eggs collected was determined volumetrically. The eggs were then added to the hatching incubators (Youshchenkov) (Dettlaff et al., 1993). The water (16-17 °C) was continuously renewed in order to provide oxygen and remove metabolites, while keeping the eggs slightly rotating by allowing moderate water flow rate

Determining Fertilization and Hatching Rates

Fertilization rate was determined according to Dettlaff et al. (1993) by the following formula:

$$\text{Fertilization rate} = \frac{\text{Total number of fertilized eggs}}{\text{Total number of eggs}} \times 100$$

For this purpose, 70-90 eggs were sampled randomly from each incubator in the time of second mitosis division which usually occurs 3-4 h after fertilization in most sturgeons. Afterward, each batch of eggs was sampled by fixing in formalin solution (5 ml formalin (37%) + 45 ml water) and the eggs development was investigated under a dissecting microscope. The eggs with four blastomeres in animal pole were considered as fertilized eggs and others without cleavage, with more than four blastomeres and damaged eggs were considered as unfertilized eggs. After 5-7 days of incubation, larvae hatched. Hatching rate is determined by counting the number of free larvae in the trough and the number of eggs that do not hatch after 24 hours (Gisbert and Williot, 2002) and the formula for the hatching rate is as follows:

$$\text{Hatching rate} = \frac{\text{Total number of hatched larvae}}{\text{Total number of fertilized eggs}} \times 100$$

Larval Collection

Larvae were collected, weighed and counted according to Dettlaff et al. (1993),

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then transferred and accommodated into rearing tanks. Continuous water exchanged was carried out after 3-4 days rearing in order to provide oxygen and remove metabolites. The tanks were cleaned to remove the dead and unhealthy larvae regularly by siphoning to avoid fungal infection.

Age Determination

to determine the age of the breeders, the sections of pectoral fin bone of them prepared according to Dettlaff et al. (1993). The number of the annual cycles (1 dark + 1 light cycle = 1 year) was counted under a dissecting microscope. The total numbers of the counted cycles are considered as the age of the fish.

Statistical

Analyses For the statistical analysis of the variables such as fertilization and hatching rates and total larvae produced one-way ANOVA, paired t-test and Pearson correlation were used followed by Duncan's test or Dunnett T3 using the SPSS software. In all cases, a minimum level of significance of $\alpha = 0.05$ was taken into consideration. All the curves and tables were processed using Microsoft Excel software.

Results

Bloodstocks' Indices (Age, Weight and Length of Female Broodstocks)

Tables1 and 2 are statistics and Kurtosis value of the data sets used in the statistical analysis and characteristics (age, weight, length and G.V.I., fertilization rate, hatching Rate, average of total larvae) of female Persian sturgeon broodstocks used in the study. There were no significant differences ($p< 0.05$) between the average age of the different treatments. Thus, it can be concluded that all the female Persian sturgeons used in this experiment were at the same age subset. All the female sturgeons in all treatments 0 10 15 and 20 ug/kg were located in the same weight and length subset (Duncan's test). There were no significant differences between the average weight and also length of the female breeders in various treatments ($p< 0.05$).As for the germinal vesicle index which is one of the most important indicators of the germinal vesicle polarization and its migration, there were also no significant differences among the Germinal vesicle Index (GVI) of the different treatments ($p< 0.05$). All the female broodstocks used in the different treatments are located in the same GVI subset (Duncan's test). The above-mentioned results indicated that each of the average age, total weight, total length and also the GVI of the different treatments were individually located at the same subset, and that the lack of the statistical differences ($p< 0.05$) related to the mentioned variables among the treatments is necessary for further analysis. On the other hand, the homogenous subset of broodstocks variables (such as age, weight, length and GVI) is a prerequisite for further statistical analysis over the other variables, for instance fertilization and hatching rates.

Fertilization and Hatching Rates

Fertilization was considered successful when embryogenesis occurred through second cleavage, morulla, blastula and final stage of organogenesis (Figs. 1, 2 and 3).

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Table 1: Mean and Kurtosis values of the data sets used in the statistical analysis of *Acipenser persicus*

	Mean	Std deviation	Variance	Kurtosis Std error
Total length (cm)	169.17	12.988	168.697	1.232
Total weight (kg)	30.29	4.509	20.339	1.232
Absolute fecundity	4766.892	4553.595	24.007	1.232
No ovum per gr.	31.17	28.051	786.879	1.232
Fertilization rate	43.33	38.97	1518.42	1.23
Hatching rate	44.450	40.762	1606.103	1.232
Average weight of each larvae (mg)	10.258	9.1924	84.501	1.232
Total No. larvae	84896.42	90777.16	84.01	1.23

Table 2: Characteristics (age, weight, length and Germinal vesicle Index (G.V.I)., fertilization rate, hatching rate, average of total larvae) of female Iranian sturgeon broodstocks

Treatments **LHRHa implantation**	**Average Total Length (cm) ±SD**	**Average Total Weight (kg) ±SD**	**Average Age (year)±SD**	**Average of Polarisation Index (PI)±SD**				**Fertilization rate (%)±SD**	**Hatching Rate (%)±SD**	**Average Total of Larvae (No)±SD**
				before implantation	**24 h after implantation**	**48 h after implantation**	**72 h after implantation**			
10µg/kg (1)	168.67±11.89	26.17±1.90	18.6±1.5	11.01±0.44	5.86±5.6	4.83±4.95	4.16±4.10	26.66±46.19	24.13±41.80	42480±73577
15µg/kg (2)	181.33±13.31	31.50±5.22	18.0±1.0	12.65±0.45	0	0	0	78.33±13.87	85.33±9.07	179434±101908
20µg/kg (3)	163.33±11.54	31.67±5.13	20.0±3.0	13.36±2.00	1.33±2.3	0	0	68.33±4.16	68.33±7.64	117671±31567
Control	163.33±12.58	31.80±4.53	18.3±1.5	12.31±0.05	11.46±0.38	11.03±0.23	10.87±0.29	0	0	0
Max. Min.	Max: 190 Min: 150	Max: 37.5 Min: 24.0	Max: 23 Min: 17	Max: 15.4 Min: 10.5	Max: 11.9 Min: 0	Max: 11.3 Min: 4.6	Max: 11.2 Min: 0	Max: 90 Min: 0	Max: 92 Min: 0	Max: 295750 Min: 0

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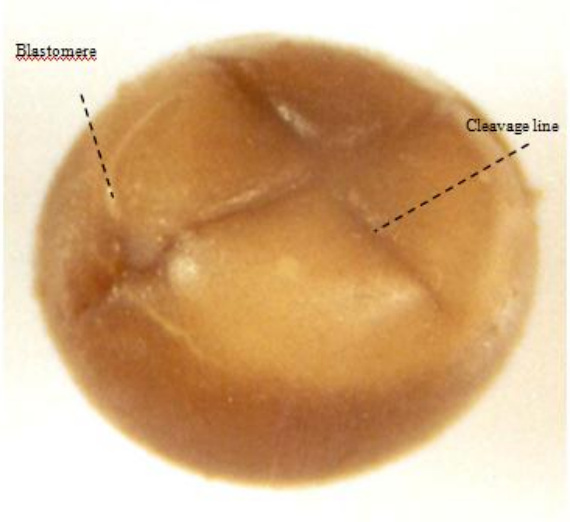

Figure 1: Second cleavage stage of Persian sturgeon

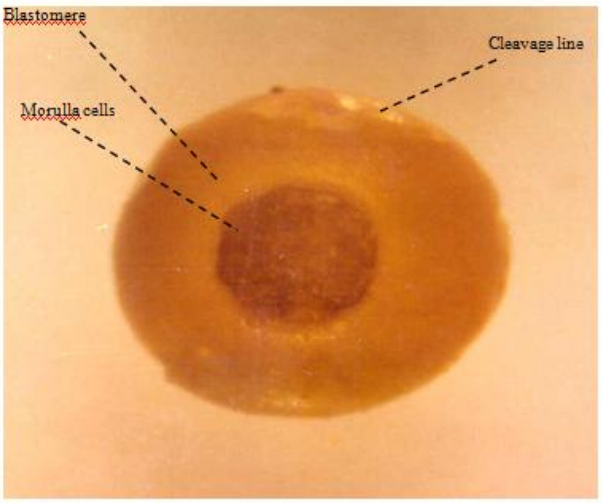

Figure 2: Morulla stage of Persian sturgeon

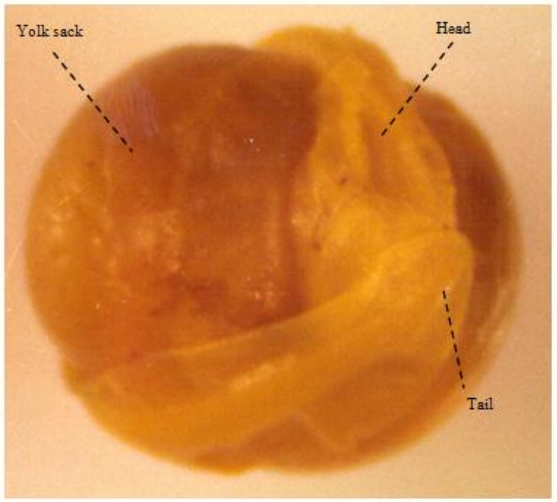

Figure 3: Organogenesis stage of Persian Sturgeon

There were significant differences in the fertilization rates of the different treatments ($p< 0.05$).The Dunnett T3 multiple comparison analysis only showed the significant differences between the average fertilization rate of control group and treatments of 15 µg/kg and 20 of µg/kg ($p< 0.05$).On the contrary, there were no significant differences between the average fertilization rates of the and treatment 1 as well as between treatment 1 and the control and also between treatment 2 and treatment 3 and likewise between treatments 2 and treatment 3.

Similarly, the statistical analysis only showed almost the same results for the fertilization rates, just like those for the hatching rates. In other words, there were significant differences between the hatching rates of various treatments with ($p< 0.05$).Based on statistical analysis, the multiple comparison of the means for the hatching rates of the different treatments

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showed significant differences between the control group and treatments 15 and 20 µg/kg ($p< 0.05$). Nevertheless, there were no significant differences between the average hatching rates of the control group and treatment 10 µg/kg and also between treatment 10 µg/kg and control, 15 and 20 µg/kg as well as between treatments 15 and 20 µg/kg.

Larval Survival

After hatching the larvae were swimming with the water current and collected. In this way the healthy larvae (Figs., 4 and 5) were separated from the dead eggs, egg shells and deformed larvae. This is an utmost important in order to avoid fungal infections of larvae and consequence larval mortality.

The statistical analysis showed significant differences between the average total numbers of larvae produced from the different treatments ($p< 0.05$). Nonetheless, the multiple comparison analysis only showed the significant differences between the control group and treatment 2 (Dunnett T3) and that there were no significant differences among the other treatments.

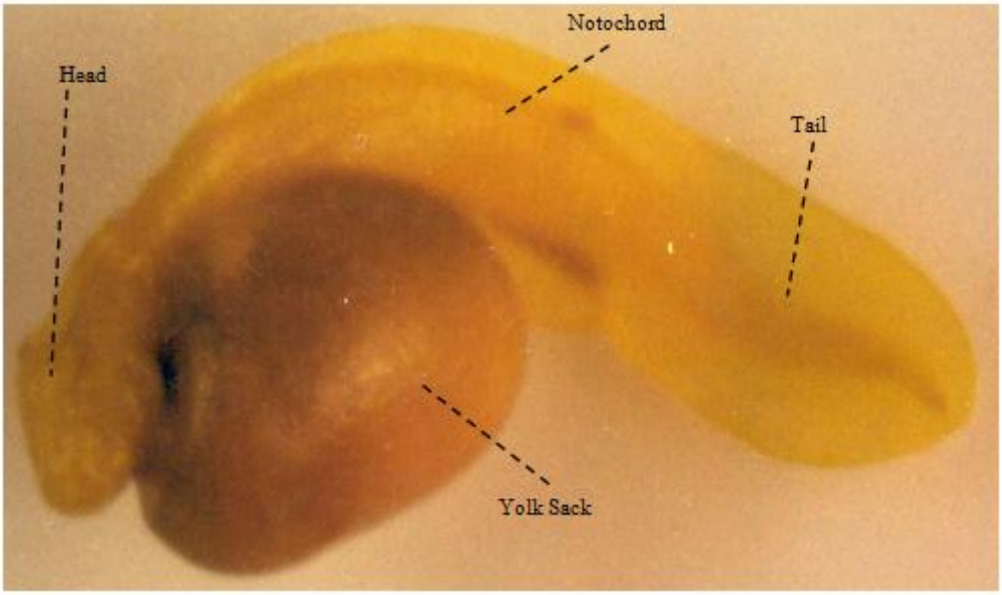

Figure 4: A one-day-old larvae of Persian Sturgeon

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Figure 5: One-day-old larvae of Persian sturgeon in larval rearing tanks

Treatments

The correlation coefficients (2-tailed) of the fertilization rates and the different treatments (control), 10, 15 and 20 µg/kg indicated a significant positive linear relationship ($p< 0.01$) amongst them, suggesting that the concentration of the implanted LHRHa had increased, and the related fertilization rates of the treatment had also increased (Figure 6, $r =0.778$). Therefore, it can be concluded that these two data sets move together.

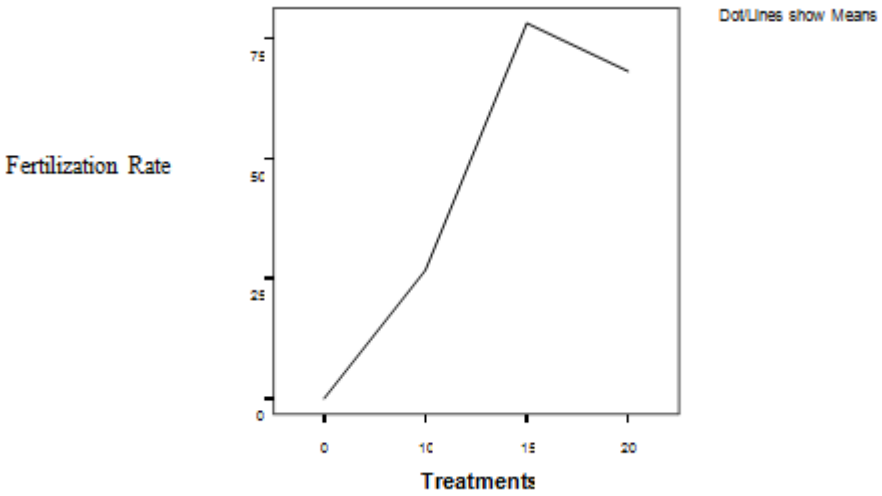

Figure 6: Fluctuation of the average fertilization rates in different treatments

The same results were also obtained for the relationship (Pearson correlation) between the hatching rates and the treatments. In other words, a significant positive linear relationship was found between the two variables ($p< 0.01$).

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Based on the data illustrated in Figure 7, the hatching rates also sharply increased (r =0.782) when the concentration of the implanted LHRHa rose.

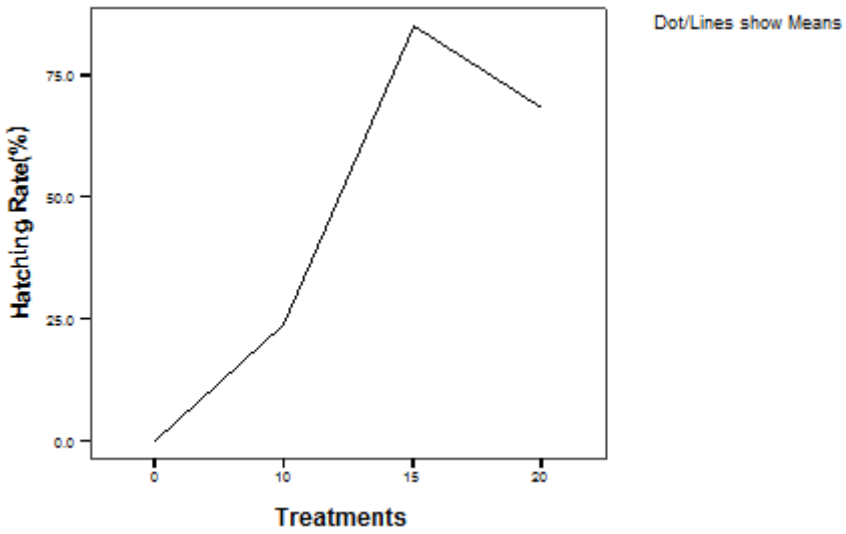

Figure 7: Fluctuation of the average hatching rates in different treatments

There was also a significant positive linear relationship between fertilization and hatching rates ($p< 0.01$). Figure 8 shows that while the fertilization rates rose, the hatching rates also sharply increased (r=.975), indicating that the two data sets move together and they are also associated with each other.

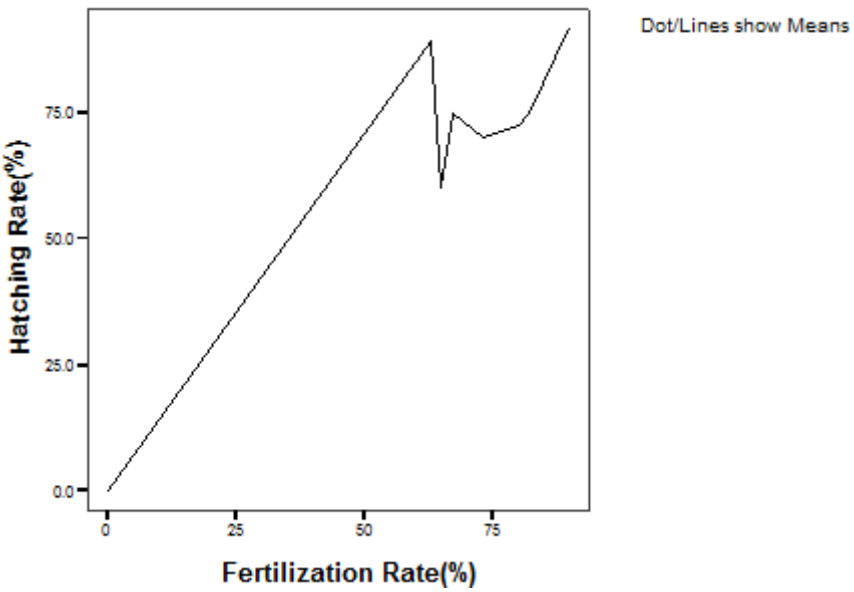

Figure 8: Fluctuation of the hatching rates with variations in the fertilization rates

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Discussion

General Aspects

The present study demonstrated that wild caught Persian sturgeon broodstocks with vitellogenic oocytes (stage 4), the Des-Gly^{10}-[D-Ala^{6}]-LHRH ethylamide administered in cholesterol pellets has a positive effect on the artificial propagation indices, such as the number of ovulated females, synchronization in oocytes ovulation, fertilization (for treatment 2 "78.33±13.87" and for treatment 3 "68.33±4.16") and hatching (for treatment 2 "85.3±9.07" and for treatment 3 "68.33±7.64") rates and total larvae produced. The LHRHa treatments accelerated the germinal vesicle migration and consequently increased the total number of ovulated oocytes in the female broodstocks of Persian sturgeons having PI higher than 10 in comparison to the control. Doroshov et al. (1982) suggested that artificial reproduction of Acipenserids presents a variety of problems most of which are related to their unusual reproductive physiology. One of the problems involves is the lack of consistent success in spawning females of apparently similar ovarian states, i.e., late stage 4.

As mentioned previously, one of the best approaches for an artificial propagation of migratory fishes is to catch matured male and female breeders from freshwater rivers. Nonetheless, the sturgeon broodstocks caught from the south-eastern part of the Caspian Sea in Iranian coastal waters are not fully matured. Moreover, the natural stocks of these fish are decreasing. Under this circumstance with the shortage of breeders, a hatchery manager must achieved high percentage of fertilization and hatching rates, through administration of suitable and modern technologies.

The Persian sturgeons with Polarization Index (PI) more than 10 used for current study were in the right stage and failed to undergo final oocyte maturation (FOM) in nature and could not complete the FOM via hormone injection in captivity. Thus, these breeders in all sturgeon hatcheries are not selected for artificial propagation to produce larvae and fingerlings but were selected for Caviar production. In this study the breeders having the right stages of FOM were treated with LHRHa hormone having slow release delivery system. We observed a complete and successful FOM and ovulation after implantation especially in treatment 3. Similar findings were observed in wild striped bass *(Morone saxatilis*), which were collected far from their spawning grounds and without the initiated FOM, required slow release implanted LHRHa for a successful ovulation (Hodson and Sullivan, 1993). According to Larsson et al. (1997) LHRHa implants were also used to significantly advance the spawning season of winter flounder (*Pleuronectes americanus*).

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In addition, hormonal treatments have also been successfully used to spawn many species that exhibit arrested reproductive development (Zohar and Mylonas, 2001). In particular, the implants containing gonadotropin releasing hormone analogue (LHRHa) have been recommended for the induction of batch spawning species (Marino et al., 2003).

Suitable concentrations of LHRHa in the sustained release delivery systems have been shown to successfully stimulate gonad development, reduce stress caused by multiple handling, and assist the broodstocks to become ready for maturation (Zohar and Mylonas, 2001). Meanwhile, the administration of the hormone in the pellets ensures a longer-term delivery than that of the injection (Crim, 1984). Although the repetitive LHRHa injection method has been effectively used in some species, the frequent handling can cause significant stress that lead to detrimental effects on the timing of ovulation, egg quality (Campbell et al., 1992) or broodstock health (Harmin and Crim, 1992., Zohar and Mylonas, 2001).

The delivery methods for administering LHRHa multiple injections or slow-release implant have similar effects on the timing of ovulation. Implants clearly have the advantage that the fish are handled only once, thus reducing stress effects. This approach has been proven to be a much studied and successful technique for spawning marine fish in captivity (Barbaro et al., 1997; Matsuyama and Chuda, 2000; Zohar and Mylonas, 2001). Sustained-release delivery systems for LHRHa also have been employed successfully for the induction of FOM, ovulation and spawning in white bass Mylonas et al., 1997.

It is worth highlighting that it is difficult to recommend precise doses of LHRHa in cholesterol implants for different sizes and species of fish. Preliminary data (Almendras et al., 1988) suggest that the threshold dose of LHRHa in a cholesterol pellet formulation is approximately 25-125 µg hormone pellets for the induction of spawning of sea bass with body weight ranging from 1 to 5 kg. According to several researchers (Sink *et al.*, 2010), in order to control, improve, and synchronize reproduction of Atlantic croakers *(Micropogonis undulats*) with a single 75 µg $kg^{-1,}$ BW LHRHa implant should be injected at 10 h of daylight and in a water temperature of 20-21◦C. Mugnier et al. (2000) indicated that in the cultivated turbot *Scophthalmus maximus*, the use of LHRHa implants is permitted under normal production conditions, whereby 56% of the females ovulate during the exploitation season, and the other fail to spawn or start ovulation late. With the use of pellets, the percentage of ovulating females can be increased to 90%. It is more difficult to select an appropriate female to induce spawning

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from a cultured population than from wild-caught brood fish. Moreover, it is quite important to obtain high quality eggs for a high hatching rate. Since female sturgeons mature slowly, breeders must wait a few years for the next breeding cycle if they fail to induce high quality eggs (Omoto et al., 2005).

Synchronization in Oocytes Ovulation

The most common problem in females is failure of the oocytes to undergo final maturation and ovulation (Donaldson and Hunter, 1983). Sturgeons are synchronous spawners. In the control treatment, none of the treated females ovulated and subsequently spawned, whereas among the females with (10 μg kg^{-1} BW) LHRHa implantation, only one fish ovulated and spawned after 50 h of implantation. In relation to the treatment with (15 μg kg^{-1} BW) LHRHa implantation, the females spawned 46-51 h post implantation, and in treatment 3, all of the treated females were found to have ovulated and spawned at the same time, i.e. at 36 h of post-implantation. Thus, the implementation of LHRHa can result in an acceleration of oocyte final maturation and ovulation in Persian sturgeon with vitellogenic oocytes.

These observations highlighted synchronization in oocytes ovulation from the induced spawning appears to be related to LHRHa dose, as indicated by the statistical analysis carried out in the present study. When implemented, the optimum dose of LHRHa leads to the best synchronization of oocyte ovulation in the Persian sturgeon, which is (20 μg kg−1 BW). Mugnier et al. (1999) suggested that cultivated turbot (*Scophthalmus maximus)* were successfully spawned using sustained-release pellets containing a gonadotropin-releasing hormone analogue LHRHa hormone. Significant synchronization of the females was obtained, and this reduced the spawning season by about half. In addition, the number of spawning females was also increased, and all sexually matured females were found to have ovulated.

Synchronisation and increases in the numbers of spawning females have also been obtained with LHRHa administration in the species showing synchronous oogenesis (Wallace & Selman, 1981), such as rainbow trout and brown trout (Billard et al., 1999). These responses have also been obtained in fish showing group-synchronous oogenesis, such as turbot (Alvarino and Peleteiro, 1993).

Morehead et al. (1998) showed the efficacy of LHRHa in inducing and synchronizing ovulation in striped trumpeter, which resulted in reliable quantities of viable eggs obtained from wild broodstock during the spawning season. LHRHa has been used to induce spawning of *Lutjanus* in three different studies. Among others, Schipp and Pitney (1995) administered 50–100 μg kg^{-1} BW LHRHa implants to induce spawning in *L. johnii*, whereas Castro and Duncan (2007) applied it to induce the spawning of caught

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matured *L. guttatus* using LHRHa implants. In those studies, the (75 μg kg^{-1} BW) LHRHa implant yielded the highest quantity and quality of spawned eggs from matured female *Lutjanus guttatus.* Meanwhile, some previous studies carried out on black seabass demonstrated that a single cholesterol–cellulose pellet, containing (50 μg kg^{-1} BW) LHRHa, successfully induced multiple spawning events over a prolonged period (Watanabe et al., 2003). A successful result was obtained for the tiger puffer (*T. rubripes*), whereby it was observed that the fish in the control group did not ovulate in captivity, while those treated with LHRHa implants (400 μg kg^{-1} BW) completed ovulation on an average of 10.5 days from the implant to ovulation (Matsuyama et al., 1997). As demonstrated by the above mentioned investigations and also by the present study on Persian sturgeons, the administration of this particular procedure could effectively impact the oocytes final maturation which tended to lead to a good synchronized oocyte ovulation, particularly at treatment 3 (36 h). However, such synchronization can be found at treatment 2 whereby all the females were found to have ovulated and spawned between 46 to 51 h after the implantation. In other groups (control and treatment), any synchronization was not found at all. Synchronization of oocyte ovulation is an important factor which can affect all other hatchery programmes, such as larval incubation, larval raising and so on. A good synchronization may indicate appropriate hormone dose administration for treatment (20 μg kg^{-1} BW) and subsequently the dose may be advisable if its application leads to proper results in the artificial propagation of these female breeders.

Fertilization and Hatching Rates

It is quite important to obtain high quality eggs for a high hatching rate. This study has indicated that the female Persian sturgeons which had been implanted with LHRHa possessed both higher fertilization and hatching rates compared to those in the control group. Among the treated female broodstocks, fish at treatment 15 μg/kg had the greatest fertilization and hatching rates compared to the others. Nonetheless, both the fertilization and hatching rates were not significantly different between fish in the experimental LHRHa implanted groups. Good egg qualities are usually defined as those exhibiting low levels of mortality at fertilization, hatch and first feeding. In Persian sturgeons, the females containing oocytes with PI range (6-8) were selected for the artificial propagation. This also means the germinal vesicle migration to animal pole in vitellogenic oocytes is an indicator to distinguish the qualified oocytes to hormonal induction. The present study has shown that the broodstocks having polarization index (PI) higher than the range (6-8) contain viable oocytes which complete vitellogenesis and

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they can be used for commercial scale production using sustained release LHRHa. According to Garcia (1989), fertilization and hatching were adversely effected in Asian seabass (*Lates calcarifer*) which were administered with high doses of LHRHa (150–300 μg kg^{-1} BW) as compared to lower dosages (4.5–75 μg kg^{-1} BW). High LHRHa doses have been shown to diminish egg quality or induce fewer ovulations in a number of other species (Marino et al., 2003). Meanwhile, the optimal dose of LHRHa has not been established for black sea bass, and implants with the actual doses as low as (7.1 μg kg^{-1} BW) have been found to be effective in inducing ovulation without decreasing fecundity or fertility. In yellowtail flounder (*Pleuronectes ferrugineus*), treatments with different LHRHa delivery systems have been observed to increase the number of ovulations, fecundity and fertilization, and hatching success (Larsson et al., 1997). A study by Ibarra-Castro and Duncan (2007) examined des-Gly10, [D-Ala6]-gonadotropin releasing hormone ethylamide (LHRHa) implant-induced spawning of recently caught wild spotted rose snapper (*Lutjanus guttatus*). The effect of implants containing 0, 25, 50, 75 and 100 μg kg^{-1} BW of LHRH-A was assessed in 10 females which had an oocyte diameter greater than 430μm. All the fish spawned except for the controls and one (100 μg kg^{-1} BW) implanted fish. The 75 μg kg^{-1} BW implant was found to have induced the spawning of eggs, with a significantly higher percentage hatch (86.1±7.5 and 87.7±5.0%, respectively), and twice as many hatching eggs kg^{-1} (BW).

Hatchery-produced white bass *(Murone chrysops)* and striped bass (M. *suxutilis)* reared to maturity in a commercial aquaculture facility were successfully spawned using the controlled-release delivery systems containing the gonadotropin-releasing hormone analogue DAla6, F'ro'[NEt]-GnRH (GnRHa). Two-year-old white bass females (with a mean weight of 0.8 1 kg) were implanted with different polymer-based, LHRHa delivery systems at doses ranging from 40 to 89 μg kg− 1 BW. For the females containing oocytes up to 720 μm in diameter, the LHRHa treatment carried out on 20 February 1994 was observed to have induced ovulation of all fish between 35 to 82 h after the treatment. The white bass eggs produced were fertilized with the sperms from the striped bass for the production of sunshine bass. An average of 294500 eggs kg^{-1}, was produced, with a mean fertility of 81.2%, 24 h survival of 46.5%, and overall hatching success of 45%. Meanwhile, the survival from the hatch day to 30 days of post-hatch was 78%, and the fry weighed between 0.07 and 0.1 g (Hutson, 2006). One of big achievements of this project is the development of a protocol utilizing fish with PI more than 10 able to spawn. Under natural condition, these fishes with

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PI more than 10 occur in high numbers and useful for breeding purposes. Table 5.1 shows the results of two treatments (2 and 3) for the endangered sturgeon fish in the past 10 years. The information presented in this table is taken from the annual census carried out by Iran Fisheries Organization.

Due to the similarity of the methods used in artificial breeding, it shows the method for LHRHa hormone implanting is far better than normal breeding. Some results for this method have been highlighted in the chapter of thesis. More importantly, the fish that were not able to breed naturally were capable of breeding with the help of this method. Furthermore, the survival of larvae and hatching is much better although not much difference is seen in the breeding stage. This is due to hormone implantation and gradual usage of hormone into the body of fish. Based on the results obtained in this study, it can be concluded that this method is a new method used in artificial breeding of *Acipenser persicus*. It is important to note that the application of the aforementioned method for fish having polarization index (PI) lower than 10 was effective because the absorbance of hormone is more efficiently done. Thus, the percentages of breeding and hatching are much higher than before. In addition, the applied method has shown the short-term effect of gradual absorbance of hormone in maturation and spawning.

Table 3: Comparison of some artificial propagation indices between treated Persian sturgeon female breeders with LHRH-A implantation and the current methods (hormone injection) at the Sturgeon hatcheries in Iran

treatments	Broodstocks responded to hormone induction (%)	Average IP	Average Fertilization rate (%)	Average Hatching rate (%)	Average No. of larvae per female fish
Treatment (2) (present study)	100	12.65±0.45	78.33±13.87	85.3±9.07	179434±101908
Treatment (3) (present study)	100	13.36±2.3	68.33±4.16	68.33±7.64	117671±3156
Fish propagated in Shahid Marjanie Centre (evidence)	81/5	8-10	65	59.6	93660

The findings of this study also suggest that this method is useful for the fish in the maturity level 4 which cannot proceed to the next stage (5). This technique may also be recommended for fish in the level of maturity lower than 4 to successfully become matured in level 5. Although this particular method is experimentally tested for sturgeon fish, the method can be implemented for other cultural fish as well. In future studies, the method can be modified with various concentrations and

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dosage for other fish in the different levels of maturity. The current acute implantation of LHRHa was able to enhance breeders to final maturation with PI higher than 10 which can't be achieved under normal condition at sturgeon's hatcheries. The results suggest that final maturation can be achieved with high fertilization and hatching rates and also best synchronization and high larval survival. Thus, this information is very useful for artificial propagation of not-fully-matured females of Persian sturgeon at sturgeon's hatcheries.

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