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Correlation Between Serum Steroid Hormones and Some Reproductive Indices in Persian Sturgeon, *Acipenser persicus*

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Abstract: The correlation between serum steroid hormones, testosterone (T), 17β-estradiol (E2) and progesterone (P) and weight and fork length of female fishes, biological characteristics of gonad (e.g. the number of eggs/gram, the number of larvae/gram, fluid egg rate, relative fecundity, gonadosomatic index, hatching success and fertilization rate) in 16 female of the migratory population of Persian sturgeon (*Acipenser persicus*) in Spring of the 2012 were determined. Results illustrated that there was no significant correlation between testosterone, 17β-estradiol and progesterone with biological characteristics of gonad ($P<0.05$). With increasing in body weight, the testosterone and 17-β estradiol concentration were increased but this increase was not significant. Also relationship between 17-β estradiol with fork length was direct, but this correlation was not significant.

Key words: *Acipenser persicus* · Steroid Hormones · Testosterone · 17β-Estradiol · Progesterone · Fertilization Rate · Gonadosomatic Index

INTRODUCTION

The Caspian Sea is the largest continental water body on earth [1], surrounded by Russia, Kazakhstan, Turkmenistan, Iran and Azerbaijan [2]. It is the habitat for the four commercial species of sturgeon (the producers of valuable black caviar) among which Persian sturgeon (*Acipenser persicus*) is one of important species that live in the southern margin of the Caspian basin [3]. Unfortunately, nowadays this fish has become an endangered species [4] due to the damages of their natural spawning environments, overfishing for meat and caviar production and also water pollution [5]. Substantial efforts were focused on the early life developmental stages of this group of fishes in order to understand how to increase their survivorship and improve hatchery efficiency [6]. Egg quality traits could be helpful for fish farmers in order to assess fingerling production, hatching management, improving rearing techniques and evaluation of the quality of fish produced. For increasing the survival rate at different stages of these fishes, it is necessary to study the potential factors affecting the growth and survival rate of incubating eggs, larvae and other larger stages [7]. Also early life stages of development are some of the most important phases of fish development, which include the replacement of embryonic adaptations and functions [6].

Sex steroids have long been recognized as key hormones regulating sexual differentiation, physiological aspects of reproduction and the development of primary and secondary sexual characteristics [8]. Both testosterone and 17-β estradiol play the major roles in oocyte development and maturation. The production of testosterone in the thecal cells of the ovarian follicle is stimulated by gonadotropins released into the bloodstream by the pituitary gland. Testosterone is converted into E2 by aromatization in the granulosa cells [9]. 17-β estradiol, controls pivotal physiological events in female reproductive cycles in all vertebrates studied to date. In many teleosts it has been reported that plasma 17-β estradiol levels increased during the vitellogenic stage but decreased during the

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maturational stage. E2 is known to induce the synthesis and release of vitellogenic protein by the liver [10]. Progesterone may directly enhance maturation through vitellogenesis stimulation or act as a precursor to Vitellogenesis Stimulating Ovarian Hormone (VSOH) [11].

The association of changes in gonadal development with plasma levels of sex steroids has proven to be a valuable tool for understanding the endocrine control of reproduction and for purposes of restocking valuable species such as sturgeon fishes. There has been an increased demand for information on all aspects of sturgeon biology and physiology [2, 12].

Studies on the valuable species reproduction assist the aquaculture industry in inland rearing and restocking specially those endangered fishes such as sturgeon fish by improving protocols for higher efficiency of egg production and larval survival and in spite of the importance of sexual hormones on fertilization process of fish, this study with the objective of investigating the correlation of serum steroid hormones (testosterone, 17β-estradiol and progesterone) with weight and fork length of female fishes and number of biological characteristics of gonad (e.g. the number of egg/ gram, the number of larvae/gram, fluid egg rate, relative fecundity, gonadosomatic index, hatching success and fertilization rate) in Persian sturgeon (*Acipenser persicus*) was carried out.

MATERIALS AND METHODS

Broodstock Preparation: In this study, female Persian sturgeon (*Acipenser persicus*) were captured at six to eight week intervals beginning in March 2011 from southeast of Caspian Sea, during their upstream migration and then transported to Shahid Margani sturgeon fish farm (Golestan, Iran) by special car in tank with oxygenation. A group of males' breeder was captured and all broodstocks of both sexes were maintained in several separate circular tanks (8 m diameter, 1 m depth, 50 m^3 volume). In this study, 16 mature female with average±standard deviation weight of 21.5±15.65 kg and fork length of 1.01 ± 81.8 meter was used. They were injected intramuscularly with acetone-dried sturgeon pituitary at water temperature in the range of 17-20°C (45-50 mg for female and 35 mg for male).

The semi dry fertilization method was used and the insemination dosage was 1 percent of egg volume for each fertilization experiment.

Incubation and Fertility Examination: After eliminating eggs adhesiveness, eggs were placed in Yushchenko incubators in running freshwater system at17-20 °C and in the presence of dissolved oxygen more than 6 ppm.

In order to calculate the fertilization rate, three hours after fertilization and 100 eggs were randomly removed and preserved in formalin 10% solution, monospermic percentage was considered only for the eggs containing four cells.

In order to determine the hatching rate of each broodstock, the number of fertilized eggs which transferred to each incubator as well as the number of larvae from each incubator, belonging to each female, calculated using the following formula: number of egg (or larvae) = number of egg (or larvae) in gram × the weight of all attaining eggs in gram that this trials was done as 3 replicates in sterile Petri dishes.

Hatching success is calculated by dividing the number of larvae by the ovum number, according to the following formula: Hatching success= number of larvae / number of ovum×100.

Gonadosomatic index was calculated using the formula GSI = Wg/W × 100, where Wg: gonad weight, W: fish weight.

For determine the fluid egg rate, the egg mass left in the body cavity of the fish was also weighed. The relative fecundity was calculated by dividing the total egg number by the total body weight.

Measurement of Serum Steroid Levels: Before the female injection with acetone-dried sturgeon pituitary, blood samples were taken from caudal vein with a nonheparinized syringe and centrifuged for 10 min at 3000×g and then serum was stored at -20°C until analyzed. Testosterone, 17β-estradiol and progesterone were measured by Enzyme-linked immune sorbent assay (ELISA) according to the procedure of Semenkova *et al*. [13]. Commercial kits for measurement of E2, T and P levels in the sturgeon serum were obtained from Tehran, Iran.

Statistical Analysis: The correlation sexual hormones and weight and fork length of female fishes, biological characteristics of the gonad (e.g. the number of egg in one gram, the number of larvae in one gram, fluid egg rate) relative fecundity and gonadosomatic index, hatching success and fertilization rate were analyzed using the bivariate correlation coefficients of Pearson (SPSS, ver. 16).

Table 1: Reciprocal correlation between sexual hormones and biological characteristics of Persian sturgeon (*Acipenser persicus)*

	Testosterone	17-β estradiol	Progesterone
Weight (kg)	0/099	0/203	-0/149
Fork length (m)	-0/006	0/186	-0/097
Number of egg (g)	0/117	-0/070	0/041
Number of larvae (g)	0/076	-0/079	0/003
Fluid egg rate	0/340	0/241	-0/050
Relative fecundity	0/324	0/136	0/092
Gonadosomatic index	0/343	0/162	0/035
Hatching success	0/084	-0/061	-0/074
Fertilization rate	0/255	0/116	0/126

RESULTS

Correlation between sexual hormones (testosterone, 17-β estradiol and progesterone) and biological characteristics of Persian sturgeon (*Acipenser persicus*) are presented in Table 1.

As shown in Table 1, progesterone was negatively correlated with four variables: weight, fork length, fluid egg rate and hatching success, but these correlates were not significant. Also relationship was the same between 17-β estradiol with the number of egg/gram, the number of larvae/gram and hatching success. In this study, testosterone with fork length had a negative relation that was not significant. With increasing in body weight, the testosterone and 17-β estradiol concentration were increased but this increase was not significant. The relationship between progesterone, 17-β estradiol and testosterone with relative fecundity, gonadosomatic index and fertilization rate was direct, but these correlates were not significant.

DISCUSSION

Artificial reproduction of sturgeon is based on the hormonal stimulation of gamete maturation [14]. The objective of induced ovulation with hormone injection in aquaculture is to produce a large supply of high quality eggs [11]. As any future industries will be reliant on artificial rearing of larvae and juveniles, it is essential to understand the reproductive physiology of valuable species such as sturgeon fishes. Three sex steroid hormones, 17β-estradiol (E2), 11-ketotestosterone (11-KT) and 17α, 20β, dihydroxy- 4-pregnen-3-one (DHP), are well established as primary estrogen androgen and progestin, respectively, in teleost fish. *In vitro* and *in vivo* assays suggest that 11-KT and E2 play primary roles in previtellogenic and growth of oocytes, respectively, whereas DHP is essential for induction of final oocyte maturation [15]. Sex receptors were identified in the early stages of a fish's gonads' development. Therefore, steroids can have an effect in early stages of the growth of fish and on gonad activity [16].

A positive correlation of 17-β estradiol, progesterone and testosterone with fertilization rate, relative fecundity and gonadosomatic index was observed in this study but these correlates were not significant. A significant positive correlation of estradiol-17β and progesterone with ovarian development was reported in the giant tiger shrimp, *Penaeus monodon*, also negative relationship between ovarian development and the level of steroid hormones was detected in the kuruma prawn, *Marsupenaeus japonicus* [17]. The inverse relationship between the gonad index and progesterone level in the ovaries of *S. mollis* was reported [10].

E2 in the serum is good bio-marker for researchers to investigate sturgeon maturity. Fujii *et al*. [18] indicated that vitellogenesis and large E2 concentrations are correlated in maturing bester (*Huso huso* x *Acipenser ruthenus*) sturgeon. Although the primary role of E2 is to aid in gonadal development, T is involved in other functions, such as positive and negative feedback control of the hypothalmo–pituitary–gonad axis and migratory behavior in sturgeons [19]. Testosterone appears to play an important role in several stages of the sexual and migratory cycles in *Acipenseridae* [20]. A number of studies have demonstrated that gonadal steroids act via a classical negative feedback loop to inhibit GTH release from the mammalian pituitary. In this regard, testosterone was shown to inhibit GTH secretion by impairing pulsatile release of GnRH. Testosterone has also been shown to stimulate GTH secretion by acting directly at the level of the pituitary.

Both negative and positive effects of gonadal steroids on GTH production have also been demonstrated in teleost species [21].

In this study, testosterone with fork length had a negative relation that was not significant. In another sturgeon species such as Siberian sturgeon (*Acipenser baeri*) the process of oocyte growth was more directly correlated with the plasma concentrations of androgens rather than either estrogen [22]. In other hand, blood samples were collected before the female injection with acetone-dried sturgeon pituitary that plasma levels of sex steroids are low prior to vitellogenesis [9].

However, Sakomoto *et al.* [23] have proposed that variations in blood parameters among fish could be affected by other variables such as the sampling technique, the capturing method, the condition of captivity and the analysis techniques.

In other hand, factors such as photoperiod, temperature, salinity and pH of the water influence egg quality [24].

It is evident that understanding the physiological indices of blood serum of *Acipenser persicus* is essential for aquaculture in Iran, because it reveals normal indices for propagation, rearing and stocking of this species.

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