

# Changes in Serum Steroid Hormones by Ovarian Development of Persian Sturgeon (*Acipenser Persicus*) during Final Maturation Induced by Hormonal Treatment

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## Abstract

In order to provide detailed information on the reproductive development of wild female Persian sturgeon (*Acipenser Persicus*), this study characterized the seasonal variation of the steroid hormones testosterone (T), 17 $\beta$ -estradiol (E2), and progesterone (P) during the reproductive cycle and their correlated with ovarian development stage. Gonad and blood of 44 specimens was taken from the wild female Persian sturgeon from May 2011 to May 2012. The results of present study showed that GSI began to increase during ovarian development and reached 23.58  $\pm$  1.08% in stage V. female in stage II (Immature) and VI (after ovulation) had a significantly lower mean serum sex steroid than all other stages ( $P < 0.05$ ). Then, under vitellogenic oocytes (stage III) sex steroids increased (5.33  $\pm$  1.06, 0.48  $\pm$  0.1 and 6.96  $\pm$  1.05 ng ml<sup>-1</sup> E2, P and T respectively) and E2 concentration was significantly higher than all other stage ( $P < 0.05$ ). At the stage IV (maturing phase), E2, P and T levels declined (1.98  $\pm$  0.48, 0.25  $\pm$  0.13 and 3.54  $\pm$  1.17 ng ml<sup>-1</sup>, respectively). T and P had surged by 24h after second injection (stage V) of pituitary preparation (PP) and then decreased sharply after ovulation.

**Keywords:** Persian sturgeon; Steroid hormones; Testosterone; 17 $\beta$ -Estradiol; Progesterone

## Introduction

Gonadotropins of fishes share many structural and functional characteristics with their mammalian counterparts [1]. It is known that FSH and LH are involved in the regulation of ovarian steroids secretion [2]. Fish FSH is believed to stimulate follicular growth in the ovary and spermatogenesis in the testis, whereas LH is involved in control of the final steps leading to ovulation and spermiation [1]. A direct relationship between plasma FSH and E2 levels [3] and between LH and P levels has been demonstrated [4].

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 [5].

Cyclical changes in the reproductive hormones of teleost fishes are widely known to occur in association with reproductive cycles. Generally, these studies have followed plasma steroid levels over the course of a reproductive season or annual cycle [6]. And have been investigated mainly to understand the mechanisms of reproductive behavior, gametogenesis, and gonadal steroidogenesis [6,7]. Reproduction provides a key to the future success of sturgeon populations, and understanding reproduction becomes ever more crucial for successful management [8].

The association of changes in gonadal development with plasma levels of steroids has proven to be a valuable tool for understanding the endocrine control of reproduction in teleosts. It is well known that, in teleosts, vitellogenesis and final oocyte maturation are regulated by gonadotropins via steroids secreted by the follicular cells surrounding the oocyte. Of these steroids, 17 $\beta$ -estradiol stimulates in turn the hepatic synthesis and secretion of vitellogenin which is accumulated in the oocytes [8].

Fish are seasonal spawners and undergo distinct annual reproductive cycles in response to environmental cues. Sexual maturity

and gonadal development is associated with increased circulating levels of gonadotropins and the steroids [9,10]. In fish, the circulating concentration of sex steroids such as testosterone and estradiol are important factors controlling the pre-ovulatory GtH surge [11].

The aim of the present work was to study the changes in the circulating levels of sex steroid in relation to the gonad development of Persian sturgeon.

## Materials and Methods

The study was conducted between May 2011 and May 2012. 44 specimens of female Persian sturgeon were captured in gillnets from southeast of Caspian Sea during year. Total weight (27.54  $\pm$  5.15) and fork length (155  $\pm$  18.04) of the fishes were measured.

The blood samples were taken from caudal vein with a nonheparinized syringe and centrifuged for 10 min at 3000 $\times$ g, and then serum was stored at -20°C until analyzed.

The captured fishes in late winter and early spring that were in stage III-IV or IV transferred to Shahid Marjani sturgeon Propagation Center in Gorgan, Iran. Two injections of sturgeon pituitary preparation (PP) (3-5mg kg<sup>-1</sup>) were used to simulate final maturation. The first injection PP (5% of total dose) was made at 10 pm and second (95% of total dose) 12 h later at 8 am hours. And 24 h after second injection the blood samples were taken from caudal vein with a nonheparinized syringe.

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Testosterone (T), 17β-estradiol (E) and progesterone (P) were measured by Enzyme-linked immune sorbent assay (ELISA) according to the procedure of Semenkova et al. Commercial kits for measurement of E2, T and P levels in the sturgeon serum were obtained from Tehran, Iran.

For determination of ovary stage in females we used histological examination. The gonad samples of females during each season were fixed in Bouin's fluid for 48h and then transferred to 70% ethanol for storage until processing for light microscopy. Paraffin sections of 4-7 μm thickness were stained with hematoxylin and eosin. The developmental stages of gonads were classified according to the system of Amiri et al. [12].

The gonadosomatic index (GSI) of female fish was calculated by dividing the ovaries weight (WG) by the whole body weight (WT) and multiplying by 100 [13].

$$GSI = WG / WT \times 100$$

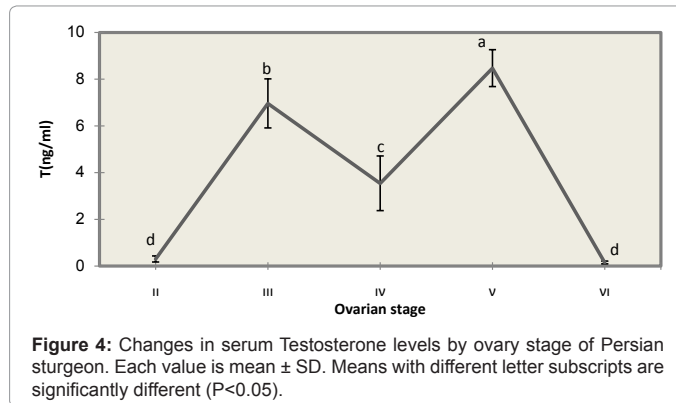
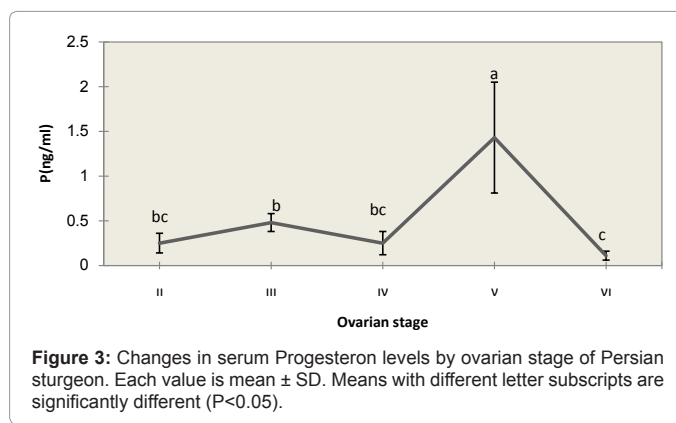
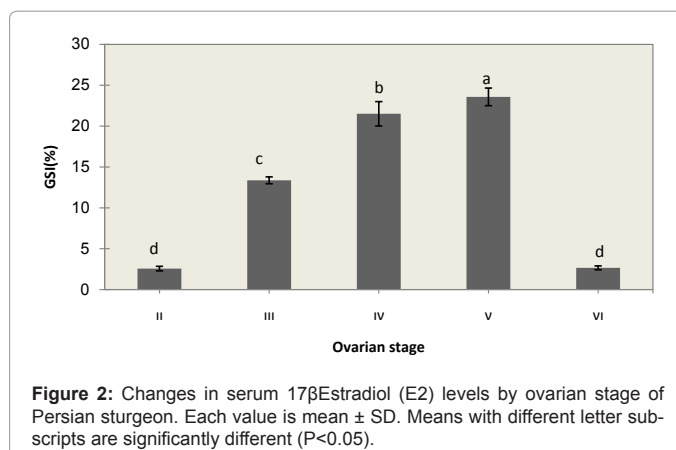
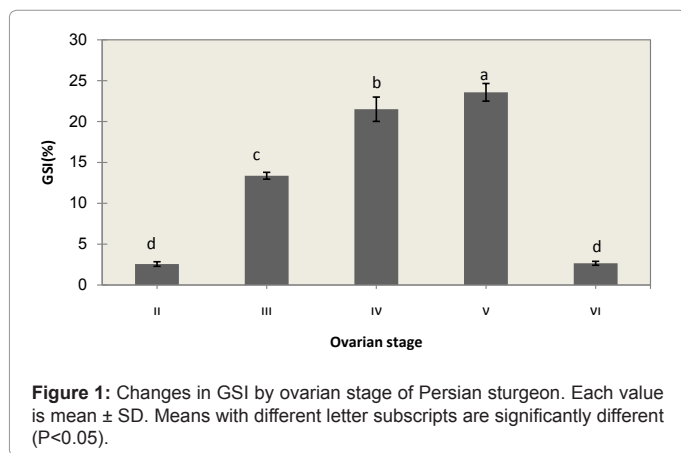
All data were expressed as means ± SD. The SPSS 16 software was used for statistical analyses. Changes in plasma levels of E, T and P were assessed by one-way ANOVA, Duncan's multiple range tests. Assignment of data correlation was done by Pearson tests, and relationships between E and T levels were examined by non-linear regression (power). The significant differences were determined at  $P < 0.05$ .

## Result

The ovary stages of Persian sturgeon, based on our previous study [14], are summarized in table 1. GSI in stage II was very small ( $2.56 \pm 0.30\%$ ) and GSI in stage III and IV increased and reached  $13.39 \pm 0.25\%$  and  $21.63 \pm 1.28\%$ , respectively (Figure 1).

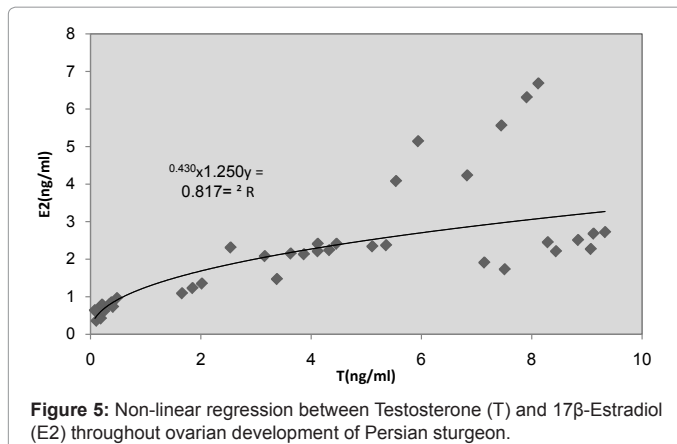
Changes in sex steroids of Persian sturgeon are shown in figures 2-4. Mean serum steroid hormones concentration were different among ovarian stages. Immature (stage II) female captured in autumn 2011, had a significantly lower mean serum sex steroid ( $0.78 \pm 0.1$ ,  $0.3 \pm 0.13$  and  $0.25 \pm 0.11$  ng ml<sup>-1</sup> E2, T and P respectively) than all other stages except VI (after ovulation). There was no significant different among mean serum sex steroid hormones at stage II and VI ( $P > 0.05$ ).

Then, under vitellogenic oocytes (stage III) sex steroids and GSI increased ( $5.33 \pm 1.06$ ,  $0.48 \pm 0.1$  and  $6.96 \pm 1.05$  ng ml<sup>-1</sup> E2, P and T respectively) and E2 concentration was significantly higher than all other stage ( $P < 0.05$ ).



Then, E2 dramatically decrease during late winter and early spring that female in stage IV and V ( $1.98 \pm 0.48$  and  $2.31 \pm 0.35$  ng ml<sup>-1</sup> respectively), and there was no significant different in levels of E2 in stages IV and V ( $P > 0.05$ ).

In this present study there was a positive correlation between E2 and T ( $R^2 = 0.81$ ,  $P < 0.01$ ) (Figure 5). There was no significant different between levels of T during stages III and IV. Also, the concentration of P don't show significant different at stages II and IV, and between III and IV ( $P > 0.05$ ). There was no significant different among P concentration during ovarian stages ( $P > 0.05$ ). But, 24 h after injection PP (stage V) the concentrations of serum P and T significantly increased ( $P < 0.05$ ). Then, after ovulation (stage VI) in late spring levels of sex steroids sharply decrease and reached  $0.53 \pm 0.11$ ,  $0.11 \pm 0.05$  and  $0.15 \pm 0.06$  ng ml<sup>-1</sup> E2, P and T respectively.



## Discussion

In the present study our results showed that the GSI was increased during the development of ovary. According to the results, correlation was found between ovarian develop and GSI in female Persian sturgeon.

Changes in serum E2 levels generally were correlated with oocyte development in the ovary and increases in GSI [15]. Jackson et al. [16], found that females at the age of 3 and 4 years exhibit gonads at the pre-vitellogenic stage, with small oocytes and very low levels of estradiol (<1 ng ml<sup>-1</sup>).

Barannikova et al. [17], found low levels of E2 in pre-vitellogenic females captured in the Caspian Sea, which increased during vitellogenesis. These results were in agreement with our study. The results show that, during stage II, due to low activity of pituitary gland, the concentration of sex steroids in Persian sturgeon was very low. Steroidogenesis is controlled by action of GTHs that activate several signal transduction pathways [18].

E2 is synthesized by cooperation of the theca and granulose cell layers surrounding the oocytes, and subsequently E2 is secreted into blood [19].

During active vitellogenesis, dopamine receptor levels decreased in the pituitary, while GnRH increased in the ovary. The balance between the stimulatory role of E2 and the inhibitory role of dopamine on GnRH activity could be one of the triggers fore early oocyte growth, taking into account that plasma E2 levels increase markedly during the course of this developmental period [20]. In a variety of species, the level of serum E2 begins to increase in accordance with the appearance of active vitellogenic oocytes, and reaches the highest levels in the tertiary yolk stage oocyte in the ovary, and sharply declines in fish with postvitellogenic and atretic ovaries [15,21,22].

For Persian sturgeon under vitellogenic oocytes (stage III), due to increasing GTH secretion from pituitary gland and its effects [23], biosynthesis of sex steroids and GSI significantly increased. Then, E2 decreased during the final stages of oocyte maturation. This result was in agreement with many works that reported E2 increases during vitellogenic phase of ovarian development in female sturgeon and decreases during the final stages of oocyte maturation [12,24]. So, E2 in the serum is good bio-marker for researchers to investigate sturgeon maturity. Fujii et al. [25] indicated that vitellogenesis and large E2 concentrations are correlated in maturing bester (*Huso huso* x *Acipenser ruthenus*) sturgeon.

Synthesis of T takes place in theca cells, which then convert T into E2, which is responsible for simulating vitellogenin synthesis in the liver and leads to oocyte growth [26]. Testosterone appears to play an important role in several stages of the sexual and migratory cycles in *Acipenseridae*. High testosterone levels were demonstrated in the Russian sturgeon at the beginning of the river period of anadromous migration, despite the different states of the sex glands in fish of the spring and winter populations [27]. In *Acipenseridae*, which are members of the cartilaginous ganoids, testosterone concentrations were the higher in both plasma and gonadal tissues. In the Russian, radioimmunological analysis and mass spectrometry showed that the serum of female Russian sturgeon contained testosterone and 11-ketotestosterone during the vitellogenesis stage; the absolute level of testosterone was three times higher than that of 11-ketotestosterone [28].

In this study, after injection PP levels of T and P were higher (stage V), while E2 were lower at this time, this results were in agreement with Brannikova et al. [29] as well as Semenkov et al. [30]. Amiri et al. [12] reported that serum Concentrations of P increased dramatically in the ovulated bester sturgeon females compared to non-ovulated individuals when fish were injected with luteinizing hormone releasing hormone analog (LH-RHA).

In several teleosts, including salmonids [31] 17α, 20β-dihydroxy-4-pregnen-3-one (17α, 20β-DP) has been identified as maturation-inducing hormone (MIH). Final maturation under hormonal treatment was followed by increased serum progesterone (P4) levels in both female and male sturgeon. In stellate sturgeon the high P4 concentration during ovulation was demonstrated by Bukovskaya et al. [28]. P4 during final maturation in sturgeon could be a precursor of the MSI and androgens. Although, the role of progesterone in supporting reproductive functions in cartilaginous gonads remains unclear [28], because P4 and at least two progestagens (17α, 20β-P and 17,20β, 21-trihydroxy-4-pregnen-3-one (17, 20βS)) exhibit high potency in stimulating germinal vesicle breakdown (GVBD) in vitro and exhibit elevated plasma concentrations at ovulation [30,32,33]. Furthermore, androgens such as T and 11-KT may also have a role during final oocyte maturation in sturgeon [32].

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