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Plasma sex steroid hormones of Persian sturgeon *Acipenser persicus* as influenced by gonad development stages and season

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Abstract

To study the impact of plasma sex steroids including testosterone (T), 17β-estradiol (E_2), progesterone (P) on gonad development stages in Persian sturgeon (*Acipenser persicus*) at different seasons, 86 female specimens were sampled and investigated. Results showed that before vitellogenesis stage (stage II), the values of T, E_2 and P were relatively low (0.25, 0.55, and 0.32 ng/ml, respectively). Nevertheless, during the vitellogenesis (stage III), the levels of T, E2 and P increased considerably up to 8.55, 4.53 and 0.52 ng/ml, respectively, and showed a significant difference, compared with those of previous stage ($P < 0.05$). In stage IV, when nucleus had migrated, level of sex steroids decreased (T = 7.44, E_2 = 2.65, and P = 0.36 ng/ml). GSI (Gonadosomatic index) at stages II, III, and IV of gonad maturity, were 2.55%, 13.11%, and 21.18%, respectively. Sex steroids therefore showed the considerable role in growth and development of gonad in Persian sturgeon and their concentrations were changed, depending on different stage of gonad maturity stage and season..

Keywords: Persian sturgeon, *Acipenser persicus*, Gonad development, GSI, Season, Sex steroids

Introduction

Reproductive biology of fish has been a widely interesting field. In teleosts, gonadal steroids are known to modulate both the synthesis and release of gonadotropins by the pituitary and influence several brain functions that are clearly responsible for gender-specific differences in the regulation of hypothalamus-pituitary-gonadal (HPG) axis (Kortner 2008). However it appears that gonadotropins do not act directly but work through the gonadal biosynthesis of steroid hormones which in turn mediate various stages of gametogenesis (Nagahama 1994). Sex steroids have two classical functions in fish development: they act as morphogenic factors during sex differentiation and as activational factors during sexual maturation (Thibaut and Porte 2004). Seasonal changes in the concentrations of circulating sex hormones and their importance for reproduction have been reported for several species of teleosts (Borg 1994; Rinchard and Kestemont 1996; Consten et al. 2002). It is known that the role of sex steroids in controlling the maturation cycle in teleosts especially during spawning times is altered by environmental or hormonal manipulation, and this has both theoretical and practical relevance (Flammarion 2000).

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Like in other vertebrates, testosterone (T) is present in female teleost fishes. Estrogens (17β-estradiol) and androgens (testosterone and 11-ketotestosterone) are known to be involved in a number of physiological functions such as sexual differentiation, ion and carbohydrate homeostasis, adaptation to stress, immune system functioning and reproduction (Dean and Sanders 1996). Barannikova et al. (1997) believes testosterone plays an important role in migration behavior of Russian sturgeon *Acipenser gueldenstaedti*. Generally, androgens appear to play a pivotal role in stimulating the growth of small ovarian follicles in vertebrates, at least in mammals and fish (Rohr et al. 2001).

Several species of sturgeon have become endangered due to the damages of their natural spawning environments and elevation of international trade in caviar. It is extremely important to understand the mechanism linking gonad development and reproductive performance in order to improve culture techniques. Although some changes in steroid hormone levels have been well documented in a number of fish species, less information is available for the effect of sex steroids and their relation with gonad maturity stages in Persian sturgeon *Acipenser persicus*. This study aimed to determine the condition of sex steroids hormones and their effects on gonad maturity stages at different seasons in Persian sturgeon.

Materials and methods

Fish and blood sampling

In this research, 86 specimens of female Persian sturgeon with the age of 12-23 years were captured from south-east of Caspian sea during the four seasons. The total numbers of samples in all seasons were 24, 8, and 54 female broods that were in stage of II, III, and IV, respectively (Table 2). Weight of samples was in the range of 18.60-43.20 kg and total length ranged from 152 to 194 cm. A group of broods belonged to male breeders were simultaneously captured from Caspian sea during reproduction season (spring) and all male and female broodstocks were maintained in several separated 50 m^3 circular tanks (8 m diameter, 1 m depth). The experiments were carried out in Rajaei sturgeon fish farm, Sari, Iran. Due to application of the proper size of gillnet's mesh by the governmental groups of sturgeon's fishermen in specific fishing areas, the breeders of stage I, was not captured.

Blood samples (10 mL) of females for analysis of sex steroid hormones were taken from the caudal vein and the blood serum was extracted by centrifugation (2500 g, 15 min) and then stored at -30 ˚C until used. The concentration of sex steroid hormones including progesterone (P), 17β-estradiol (E2) and testosterone (T) were analyzed by Radio immunoassay methodology as described by Fostier et al. (1982). The levels of testosterone (T) and progesterone (P) were measured by Kavoshyar Co**.** kits (Tehran, Iran) and 17β-estradiol (E2) was determined by Orion Diagnostica kits (Espoo, Finland).

Experimental procedure

The number of 41 ripened fishes in stage IV in the spring with the polarization index (PI) less than 7% (Dettlaff et al. 1993) was injected intramuscularly with acetone-dried sturgeon pituitary according to water temperature in the range of 17-20 ˚C (45-50 mg for female and 35 mg for male). For measuring GV (germinal vesicle) position by PI, a sample of 15-20 eggs for each female were boiled for 2 min and were cut along their animal-vegetal poles axis and observed under a dissection microscope with a micrometer eyepiece (Olympus, Tokyo, Japan). The position of the GV was estimated either visually or by calculating the oocyte PI ($PI = a/A \times 100$, where a: distance between GV and cell membrane, and A: diameter of oocyte along animal-vegetal axis, Dettlaff et al. 1993). During the study, the variables including fertilization rate, incubation survival rate, larvae survival rate during yolk sac absorption (before feeding), and larvae survival rate after active feeding were determined for each female broods for 41 mentioned breeders in two groups. Group I belonged to female broods (n = 19) having fertilizing rate less than 50% and group II were the female broods (n = 22) with more than 50% fertilizing rate. Therefore,19 females did not show acceptable fertilizing rate and only considered for comparative variables evaluation with 22 proper ones (Table 3). Female's ripening was examined according to Dettlaff et al. (1993) and observed every one hour after the first examination. At 30 min before the expected time of fertilization, sperm of each male was collected with 50 mL polyethylene syringes separately and its quality was assessed by means of density observation and motility test and stored in a refrigerator at 4 ˚C.

Before each insemination, the excess of coelomic fluid was removed by pouring the eggs onto a screen suspended over a beaker. The eggs were then inseminated with milt (1 female *vs*. 2 males) and after eliminating eggs adhesiveness, eggs were placed in Yushchenko incubators in running freshwater system at 17-20 ºC and dissolved oxygen was more than 6 ppm.

Three hours after fertilization at 17-20 ˚C, 100 eggs were randomly removed and preserved in 10% formalin. In calculate the fertilizing rate, the monospermic percentage was considered only for the eggs containing 4 cells (Dettlaff et al. 1993). Also, amount of larvae and malformed larvae were measured after 4 days incubation period in incubators and dead eggs were counted daily.

Gonadosomatic index (GSI) was calculated for each gonadal stage using the formula GSI = Wg/W × 100, where Wg: gonad weight, W: fish weight. For histological studies, the gonads fixed in 10% formalin after different processing steps and gonad slices were stained by hematoxylin-eusin (magnification ×56). All other chemicals were of the highest commercially available grade.

In order to measure survival of larvae during yolk-sac absorption, 300 larvae were randomly sampled from each female (at the middle of hatching time) and reared separately in small plastic tanks (20 l volume) with running water (0.5 l/min) and stable temperature (18±2 ˚C). In every morning, the excreted materials were discharged by siphoning and the mortality rate for every tank was recorded. At the beginning of the exogenous feeding, larvae were fed 6 times per day by artemia nauplii (*Artemia urmiana*) with the rate of 30% body weight per day and reared for 2 days.

Statistical analysis

The distribution of data for determination of normality was tested by the Kolmogorov–Smirnov test. Two group's data was analyzed by unpaired *t*-test and in the case of more than two groups, data were analyzed by one-way analysis of variance (ANOVA). Duncan's multiple range test was applied to determine the significance of the differences among means at $P < 0.05$ by SPSS 16.

Results and Discussion

Gonadosomatic index (GSI) in stage II was very small (2.44%) and GSI in stage III and IV significantly increased and reached 13.11% and 21.18%, respectively ($P < 0.05$, Table 1). The rapid elevation in serum gonadal steroids in Persian sturgeon from stage II to stage III was highly related with the increases in GSI.

Table 1. Values of T, E2, P, and GSI in female Persian sturgeon over stages II to IV[*]

Stages	T (*n*)[**] ng/ml	E2 (*n*) ng/ml	P (*n*) ng/ml	GSI (*n*) %
II	0.25±0.14[b] (24)	0.55±0.31[c] (24)	0.32±0.18[b] (17)	2.44±0.55[c] (17)
III	8.55±2.41[a] (8)	4.53±1.61[a] (8)	0.52±0.08[a] (8)	13.42±2.20[b] (8)
IV	7.44±5.70[a] (52)	2.65±1.45[b] (52)	0.36±0.21[b] (52)	21.14±5.49[a] (44)

[*]Data are expressed as means ± SD.
[**] *n* = Number of samples.

Biosynthesis and secretion of sex steroids and growth of oocytes were dependent on the production and concentration of gonadotropin hormones. During stage II, due to low activity of pituitary gland, the concentration of sex steroids in Persian sturgeon was very low (T = 0.25, E_2 = 0.55, and P = 0.32 ng/ml). In this stage, oocyte of samples was very small (0.350–0.450 mm) and GSI percentage was the lowest (2.44%, Table 1). The results of sex steroids being in less value in the female over stage II was in agreement with other reports. Amiri et al. (1996) mentioned that serum E_2 level was constantly low (less than 0.6 ng/ml) in immature group of the bester (a hybrid of sturgeon). They also reported that T value in immature group was low (5-15 ng/ml), but it was higher than our results. Orian et al. (1998) concluded that in immature group of *trichiurus lepturus*, E_2 level was low and had significant difference ($P < 0.05$), compared with vitellogenic group. Murayama et al. (1994) found that *Sardinops melanostictus* had the changes in plasma E_2, which were in close association with the ovarian development and it was lowest at the yolk vesicle stage. Dahle et al. (2003) reported that plasma levels of T did not differ significantly from those of stage I to V in female Atlantic cod *Gadus morhua* L. but low plasma levels of E2 were observed in immature. E2 in most cases produced by the ovary under the influence of gonadotropins was introduced into the vascular system and stimulated the hepatic synthesis and secretion of vitellogenin (Nagahama 1994).

Table 2. T, E2, P, and GSI in female Persian sturgeon over stages II to IV in different seasons*

Stages	Parameters	Summer	Autumn	Winter	Spring
II (n = 24)		n = 8	n = 6	n = 10	
	T (ng/ml)	0.25±0.11[a]	0.34±0.17[a]	0.19±0.12[a]	-
	E2 (ng/ml)	0.37±0.25[b]	0.86±0.24[a]	0.50±0.25[b]	-
	P (ng/ml)	0.30±0.09	BDL[**]	0.36±0.22	-
	GSI (%)	2.28±0.59[a]	2.78±0.56[a]	2.30±0.43[a]	-
III (n = 8)		n = 2	n = 6		
	T (ng/ml)	9.95±5.30	8.09±1.23	-	-
	E2 (ng/ml)	2.48±0.44	5.22±1.17[***]	-	-
	P (ng/ml)	0.60±0.01	0.50±0.08	-	-
	GSI (%)	11.45±2.89	14.08±1.74	-	-
IV (n = 54)		n = 6	n = 12	n = 14	n = 22
	T (ng/ml)	10.67±5.37[a]	5.16±2.19[b]	12.69±6.13[a]	4.26±3.53[b]
	E2 (ng/ml)	1.66±0.14[c]	3.29±0.86[b]	4.52±0.68[a]	1.43±0.46[c]
	P (ng/ml)	0.51±0.12[a]	0.33±0.16[a]	0.47±0.20[a]	0.27±0.21[b]
	GSI (%)	22.55±5.12[b]	15.39±1.34[c]	15.45±1.61[c]	25.40±2.99[a]

*Data are expressed as means ± SD.
BDL = below detection limit. * Significant at $P < 0.01$ by t-test between two groups of summer and autumn for E2.

For Persian sturgeon under vitellogenic oocytes (stage III), due to increasing GTH secretion from pituitary gland and its effects, biosynthesis of sex steroids, GSI and oocyte diameter significantly increased and sex steroid hormones showed the highest values (T = 8.55, E2 = 4.53, and P = 0.52 ng/ml). Rosenblum et al. (1987) found that *Ictalurus nebolosus* had the increased amount of T and E_2, which coincided with GSI enhancement. Frantzen et al. (1997) reported the females of *Salvelinus alpinus* which had commenced vitellogenesis showed significantly higher levels of T and E_2 in comparison with those female's ovaries containing no vitellogenic oocytes; gonadal growth was accompanied by increases in plasma levels of E_2 and T which peaked at 11 and 71 ng/ml, respectively. Those values were higher than those of our results. Plasma E_2 increased along with the yolk accumulation (about 3ng/ml) and reached the highest value at the tertiary yolk globule stage of *Sardinops melanostictus* (Murayama et al. 1994). E_2 and P of *Trichiurus lepturus* increased in stage III and reached 3 and 1.5 ng/ml, respectively (Orian et al., 1998). Also, Barannikova et al. (1997) indicated that T, P and E_2 levels in Russian sturgeon (*Acipenser gueldenstaedti*) were 16.7, 3.07 and 1.02 ng/ml, respectively.

Aromatase activity in granulosa cell layers increases during vitellogenesis and decreases rapidly in association with the ability of the oocyte to mature in response to gonadotropin. This decrease in aromatase activity appears to be coincidental with the decreased ability of intact follicles to produce E_2 in response to gonadotropin (Nagahama 1994). The increased plasma levels of E2 with increasing ovarian development are usually explained by the role of E2 as the main promoter of vitellogenesis. For Persian sturgeon in stage IV, the levels of E_2, P, and T decreased. This result was in agreement with many works. Decreases in E_2 and P were significant but no change in T was observed. Orian et al. (1998) indicated that P and E_2 levels decreased in stage IV in *Trichurus lepturus*. Rosenblum et al. (1987) reported that plasma T and E_2 levels of *Ictalurus hebulosus* dropped prior to the spawning period. *Salvelinus alpinus* showed the level of E_2 and T dropped during final maturation and ovulation (Frantzen et al.,1997). T levels in our study were in agreement with Amiri et al. (1996) who found that it remained at high level. T production was not decreased during postvitellogenic period (Nagahama 1994). However, Ceapa et al. (2002) reported that T reached 673.9 ng/ml in female stellate sturgeon (*Acipenser stellatus*) during spawning migration. For a study carried out by Dahle et al. (2003), E2 increased during gonadal development and reached the maximum plasma levels during spawning in Atlantic cod *Gadus morhua* L.

The Persian sturgeon tended to show the major physiological changes in stages III and IV and there were the fluctuations in sex steroid hormones under different seasons especially for stage IV (Table 2). In the stage IV, the highest level of P was measured in summer and winter (0.51 and 0.47, respectively, Table 2), whereas progesterone level was reported to be higher in spring in Russian sturgeon (*Acipenser gueldenstaedti*) (Barannikova et al. 1997).

Throughout the pre-spawning months in winter, E2 and T levels were high in Persian sturgeon and after the spawning period in summer, sex steroid hormones levels increased significantly ($P < 0.05$) for T from 4.26 to 10.67 ng/ml and for P from 0.27 to 0.51 ng/ml (Table 2).

Table 3. Sex steroid hormones and other variables in two fertilization rate's groups (group I, < 50%, $n = 19$; group II, > 50%, $n = 22$) in Persian sturgeon.*

Variables	Fertilization rate Group I (< 50%)	Group II (> 50%)	*t*	*P*-value
T (ng/ml)	1.70±1.55	4.26±3.5	-2.91	0.006**
E2 (ng/ml)	0.93±0.5	1.43±0.4	-3.28	0.002**
P (ng/ml)	0.33±0.1	0.27±0.2	1.11	0.270
GSI (%)	23.45±4.2	25.40±2.9	-1.53	0.135
Survival rate in incubation (%)	13.90±21.8	73.65±30.4	-6.70	0.000**
Larvae survival before feeding (%)	81.15±7.1	89.24±5.1	-3.30	0.003**
Larvae survival after feeding (%)	90.30±5.6	90.70±6.1	-0.15	0.876
Polarization index for GV (%)	6.60±0.5	6.60±0.7	-0.01	0.987
Fertilization rate (%)	24.64±18.0	79.28±11.5	-11.68	0.000**

*Data are expressed as means ± SD.
**Significant at $P < 0.01$.

The increasing gonadosomatic index (GSI) was positively correlated with an increase in plasma vitellogenin (Fitzpatrick et al. 1986). This was in accordance with our results for all steroids from stage II to stage III (Table 1), however, there was a decrease of sex steroids from stage III to stage IV.

According to Table 3, the levels of T and E2 were significantly higher in female broods that achieved the fertilization rate more than 50% (group II), whereas the lower than 50% was found in group I. One of the major findings of the present study was that the secretion of T and E2 played an important role in success of reproduction procedure in Persian sturgeon. Prior to vitellogenesis (previtellogenesis), androgens may play an integral role in the regulation of oocytes (immature eggs) growth. In group I (Table 3), long time of the female broods in gillnets and additional manipulation in transporting the breeders to reproduction farms might induce stress condition and consequently led to deformation of natural hormone regulations and finally decreased the efficiency of reproduction. Also, the values of P, GSI, and polarization index for GV position did not differ significantly ($P > 0.05$) between two groups (Table 3). As a conclusion, the results of this study revealed that sex steroids had the important role in growth and development of oocytes in Persian sturgeon and their concentrations were governed by gonad maturity stages and seasons.

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References

Amiri BM, Maebayashi M, Hara A, Adachi S, Yamauchi K. 1996. Ovarian development and serum sex steroid and vitellogenin profiles in the female cultured sturgeon hybrid the bester. J Fish boil 48: 1164-1178.

Barannikova IA, Bayonova LV, Saenko II. 1997. Dynamics of steroid hormones in sturgeon (*Acipenser gueldenstaedti*) with different characteristics of gonads at the beginning of an anadromous migration to the Volga. J Ichthyol 37(4): 312-318.

Borg B. 1994. Androgens in teleosts Wshes. Comp. Biochem. Physiol. C: Toxicol Pharmacol 109: 219–245.

Ceapa C, Williot P, Le Menn F, Davail-Cuisset B. 2002. Plasma sex steroids and vitellogenin levels in stellate sturgeon (*Acipenser stellatus* Pallas) during spawning migration in the Danube River. J Appl Ichthyol 18: 391-396.

Consten D, Lambert GL, Komen H, Goos HG. 2002. Corticosteroids affect the testicular androgen production in male common carp (*Cyprinus carpio* L.). Biol Reprod 66: 106-111.

Dahle R, Taranger GL, Karlsen O, Kjesbu OS, Norberga B. 2003. Gonadal development and associated changes in liver size and sexual steroids during the reproductive cycle of captive male and female Atlantic cod (*Gadus morhua* L.). Comp Biochem Physiol - Part A 136: 641–653.

Dean DM, Sanders MM. 1996. Ten years after: reclassification of steroid responsive genes. Mol Endocrinol 10(12): 1489-1495.

Dettlaff TA, Ginsburg AS, Schmalhausen OI. 1993. Sturgeon fishes, developmental biology and aquaculture. Springer Verlag, Berlin Heidelberg, 300 pp.

Fitzpatrick MS, Van Der Kraak G, Schreck CB. 1986. Profiles of plasma sex steroids and gonadotropine in coho salmon, *Oncorhynchus kisutch*, during final ovarian maturation. Gen Compar Endocrin 62: 437-451.

Flammarion P. 2000. Induction of fish vitellogenin in testicular structure: preliminary results of estrogenic effects in chub. Ecotoxicology 9: 127-135.

Fostier A, Billard R, Breton B, Legendre M, Marlot S. 1982. Plasma l-oxotestosterone and gonadotropin during the beginning of spermiation in rainbow trout (*Salmo gairdneri* R.). Gen Comp Endocrinol 46: 428-434.

Frantzen M, Johnsen HK, Mayer L. 1997. Gonadal development and sex steroids in a female Arctic charr broodstock. J Fish Biol 51: 697-709.

Kortner TM. 2008. The role of androgens on previtellogenic oocyte growth in Atlantic cod (*Gadus morhua*): Identification and patterns of differentially expressed genes in relation to stereological evaluations. Ph.D thesis, Norwegian University of Science and Technology, Trondheim, Norway. 72 pp.

Lokman PM, George KA, Divers SL, Algie M, Young G. 2007. 11-Ketotestosterone and IGFI increase the size of previtellogenic oocytes from shortfinned eel, *Anguilla australis*, in vitro. Reproduction 133(5): 955-67.

Murayama T, Shirashi M, Aoki I. 1994. Changes in ovarian development and plasma levels of sex hormones in the wild female Japanese sardine (*Sardinus melanostictus*) during the spawning period. J Fish Biol 45: 235-295.

Nagahama Y. 1994. Endocrine regulation of gametogenesis in fish. Int J Dev Biol 38(2): 217-229.

Orian S, Parivar K, Yekrangian A, Hosseinzadeh H. 1998. Sex hormones changes in the reproductive cycle of female *Trichiurus lepturus*. Iranian J Fish Sci l7(2): 49-65.

Rinchard J, Kestemont P. 1996. Comparative study of reproductive biology in single-and multiple-spawner cyprinid fish. I. Morphological and histological features. J Fish Biol 49: 883-894.

Rohr DH, Lokman PM, Davie PS, Young G. 2001. 11-Ketotestosterone induces silvering related changes in immature female short-finned eels, *Anguilla australis*. Comp Biochem Physiol 130(4): 701-14.

Rosenblum PM, Pudney J, Callard P. 1987. Gonadal morphology enzyme histochemistry and plasma steroid levels during the annual reproductive cycle of male and female Brown bulhead cat fish *Ictalurus nebuclosus*. J Fish Biol 31: 325-341.

Thibaut R, Porte C. 2004. Effects of endocrine disrupters on sex steroid synthesis and metabolism pathways in fish. J. Steroid Biochem. Mol Biol 92: 485–494.