

Seasonal Fluctuations of Steroid Hormones in Beluga (*Huso huso*) Cultured in Brackish Water Medium

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Abstract: This experiment was carried out during a period of one year on blood sexual hormone of 74 four years old Beluga with initial weight of 11kg held in 8 round cement ponds in brackish water with salinity of about 13.5 ppt. Fishes were biopsied at the beginning of the experiment in order to determine sexuality and gonadic stage. Blood sampling was performed from caudal vessels using none-heparinized syringes three times during different seasons (fall, winter and spring). Storage, centrifuging and pipetting of the samples were carried out at 4°C. Plasma for further assays was stored at -20°C. Testosterone (T), Estradiol (E2) and Progesterone (P) levels were measured by Radio Immune Assay (RIA). Test of profile was done in duplicate. Data were analyzed using SPSS software. The significant differences were determined in 5% level. Statistical results showed a significant elevation on measured factors in both sexes ($p>0.05$) (especially in males). In males, testosterone (T) depends on gonadic stage, had significant elevation; but in females, there was no significant increasing in estradiol (E2) and progesterone (P) which probably resulted from their ages. In males, T level in stages of II, II-III, III, IV, was 5.5, 4.4, 6.3, 2.6 ng/ml, respectively; it means that amount of T increase by development of gonad until III stage, then suddenly decrease in stage IV. Quantities of P and E2 were measured only in females. In these fishes, amounts of P and E2 in stages of II, II-III, were 0.12 & 0.13 and 1.455 & 0.81 ng/ml respectively, that showed increasing of P and decreasing of E2 by gonad progress. According to the results, it was demonstrated that sexual hormones are affected by season, environmental conditions and gonadal stages. So, brackish water as a new medium culture is effective in reduction maturity time in beluga.

Key words: Steroid hormones • Gonadic stage • Gonad development • Beluga • Brackish water

INTRODUCTION

Many parameters such as environmental, nutritional, genetic and endogenous factors affect fish growth. However, while many studies have examined the impact of these factors upon growth rate, the interrelationship between genetic determinism of growth and endocrine status in fish remains poorly understood. From the view point of pisciculture, it is important to establish whether growth rate is related to endogenous levels of growth promoter hormones, as they are likely to be under the same degree of genetic control and hence could be selected for in-breeding programs.

The establish relationship between different body tissues is very vital. Among this relationship, hormones have important role. Hormones are regulators which affect cells metamorphism. Hormones are not beginner of reaction as many physiological actions maybe carry out without them. Hormones divide to three major groups on

the base of their chemical construction [1]. steroid hormones, peptide hormones and amino acid products. Estrogen and progesterone produced by ovary and testosterone by testis. In addition, upper kidney gland produces some steroid hormones. Lying cells in response to LH produce testosterone. Secretion of gonadotropin from pituitary affect by gonad steroid hormones via a feed back mechanism. Overall, steroid hormones produced by upper kidney layer, ovary and testis that have similar embryonic origin.

Results from studies indicated that annual fluctuation of hormones is in relation to reproduction, feeding and growth cycle of fishes [2]. Increase of 17β estradiol, progesterone and cortisol during maturity was reported in many teleost fish [1]. Beluga is potentially the commercially most important species in the word for caviar production, but apart from extensive studies on physiological issues [2,3], little is known on sex steroid changes and gonadal development stages of this fish [4].

Gonads steroid hormones affect on secretion of gonadotropin from pituitary via a feed back mechanism. Since these hormones active before maturity [4].

Most of studies on steroid hormone were carried out in maturity and harmonious with final maturity of gonad hormone fluctuation had examined only in short time. However, with awareness to seasonal fluctuation and effect of environmental condition before maturity, it can be obtained the beneficial information about: suitable time of hormonal injection, creation of premature populations and decrease of long time maturity in this valuable fishes. Therefore, in the present study, the profile of seasonal fluctuation of steroid hormones was examined before maturation.

MATERIALS AND METHODS

This research was carried out in Inland Salt Water Fishes Research Station, Bafgh, Iran for one year (summer 2005 to spring 2006). This study, was performed in eight round roofed 30 tons cement ponds, which equipped with water distribution system and central aeration. Required water supplied through a semi-deep well with constant salinity of about 13.5 ppt. 72 four years old grate sturgeon were selected for this study, with mean weight of about 11kg from a culturing population in an brackish water earth pond and transported to the cement ponds. Fish were fed by BFT pellet food in protein and energy levels of 40% and 4500 kcal/kg, respectively. Fishes were biopsied at the beginning of the experiment in order to sexuality and gonadic stage determination. Histological preparation was stained with hematoxyline & Eosin method [5] in physiology and biochemistry Department of International Research Institute of Sturgeons (Rasht, Iran).

Blood sampling was performed from caudal vessels using none-heparinized syringes three times during fall, winter and spring seasons. 4-5 CC blood extracted from each fish. Storage, centrifuging and pipe ting of the samples were carried out at 4°C. Plasma for further assays was stored at -20°C. Testosterone (T), Esteradiol (E2) and Progesterone (P) were measured by Radio Immune Assay (RIA). The test of profile was done in duplicate. Data were analyzed using SPSS software. The significant differences were determined in 5% level.

RESULTS

Sexuality Determination: Results from biopsy indicated that from the whole of fishes, there was 57% male and

43% female. According to gonadic studies of biopsied fishes, 4% of males were in I, 11% in I to II, 60% in II, 12.5% in II to III and 12.5% in III to IV stage and about females, they were 23% in I to II, 60% in II and 17% in II to III stage.

Testosterone (T): Statistical analysis showed that the level of T differ between male and female with strong significant difference (sig = 0.00, f = 127.3) and was more in males. But, in different seasons significant difference was not observed (sig = 0.94, f = 0.062) (Fig. 1). The highest of T in males was in fall (5.33 ± 4.4 ng/ml), the lowest was in winter (5.06 ± 2.4 ng/ml) and the average was 5.22 ± 3.2 ng/ml. In females also the highest of T was in fall (0.21 ± 0.21 ng/ml), the lowest was in winter (0.1 ± 0.00 ng/ml) and the average was 0.14 ± 0.13 ng/ml.

In addition, T in different sexes had significant correlation with gonadic index (sig = 0.01, r = 0.5) (Fig. 2) and would be consider T as index of Gonadic growth in males. Histological results showed that in males, amount of T in stage II, II-III, III and IV was 5.5, 4.4, 6.3 and 2.6ng/ml, respectively and in females, was 0.10 and 0.16 ng/ml in stage II and II-III, respectively.

Progesterone (P): Amount of P was measured only in females. Statistical results showed that there was no significant difference in different seasons (sig = 0.064, f = 3.48) (Fig. 3). The highest level of P was seen in winter (0.66 ± 0.06 ng/ml), the lowest was observed in spring (0.12 ± 0.04 ng/ml) and the average was 0.37 ± 0.41 ng/ml. There was no significant correlation between gonadic indices. In addition, histological results demonstrated that in stage II and II-III respectively amount of P was 0.12 and 0.13 ng/ml.

Estradiol (E2): Level of E2 also was measured only in females. Statistical analysis showed significant difference during different seasons (sig = 0.00, f = 16.95). The highest amount of E2 was in fall (5.51 ± 2.6 ng/ml), the lowest was observed in winter (1.18 ± 0.82 ng/ml) (Fig. 4) and the average was 2.63 ± 2.6 ng/ml. There was no significant correlation between gonadic indices.

Results also showed that in stage II and II-III amount of E2 was 1.45 and 0.81 ng/ml, respectively. According to the results, amount of steroid hormones were affected by sexuality, so quantity of T in males and E2 & P in females were more (because of low concentration of P & E2 in males, these hormones measured only in females). It also was observed that only E2 affected by season.

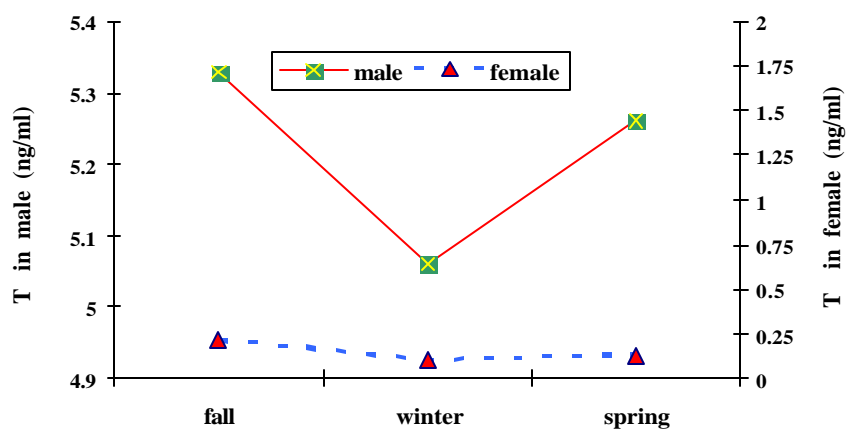


Fig. 1: Change of serum Testosterone in different seasons

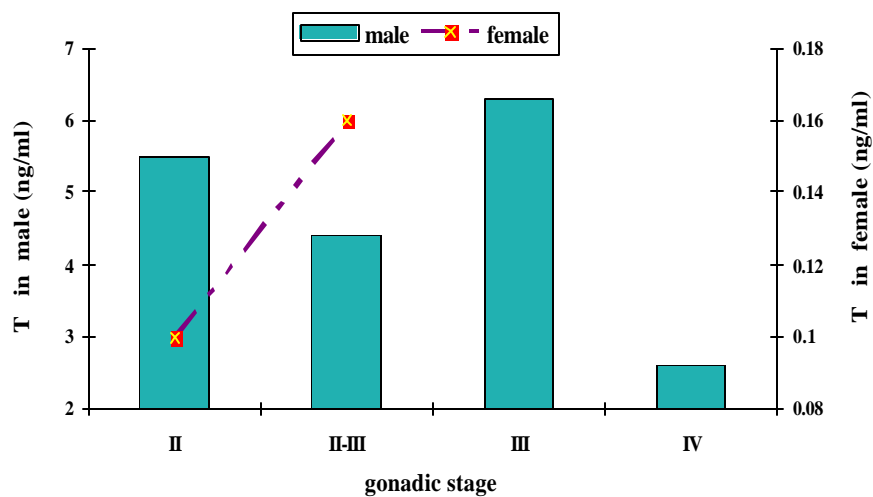


Fig. 2: Change of serum testosterone in gonadic stage

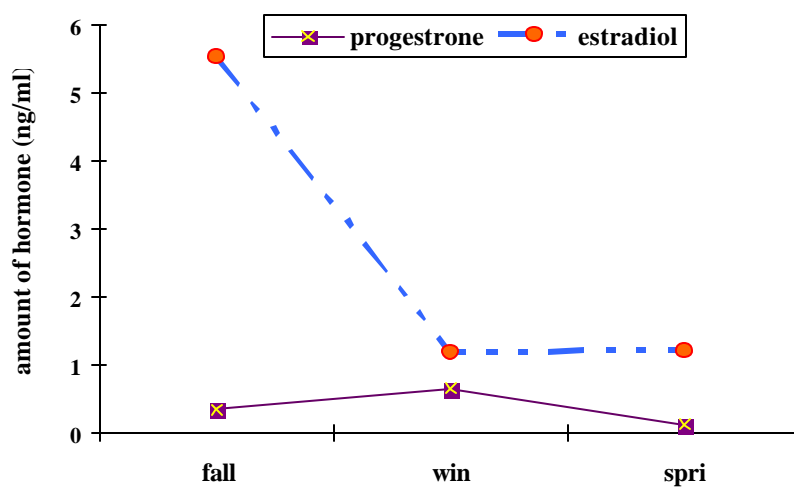


Fig. 3: Change of serum estradiol and progesterone in different season

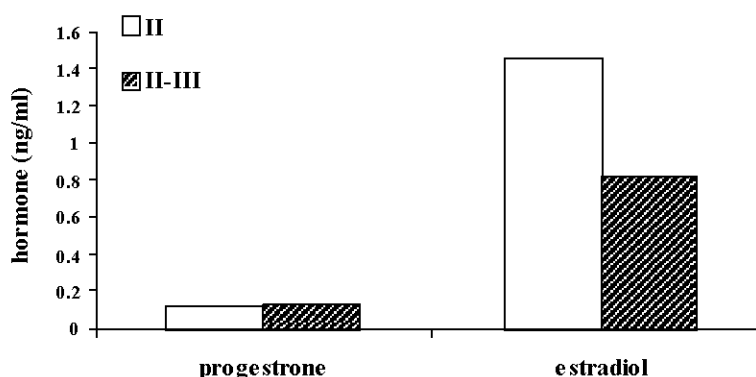


Fig. 4: Changes of serum progesterone and estradiol in different gonadic stages of females

DISCUSSION

Hormonal changes are depended on the alternation of environmental temperature, so in suitable temperature for spawning, amount of hormones will be increased [6]. Fluctuation of sexual hormones in annually, monthly and diurnal cycle in teleost fishes was proved [3].

Results showed that in males by progressing in gonadic stage until stage III, level of T increased and then in stage IV, intensely decreased. In females with progression of gonadic stage, quantity of P increased, but in contrary level of E2 decreased. This entire level is because of gonad formation that affects hormones level.

Regarding to direct correlation between secretion of sexual steroid hormones and development of gonad with synthesis and amount of gonadotropine hormones, in previtellogenic stage and stage II that oocyte is little and also separating animal and vegetable pole is not possible, amount of sexual steroid hormones is slight [7]. Therefore, about immature fishes in this study, levels of hormones were low.

Yousefian [4] examined fluctuations of E2 & T in young Great sturgeon cultured in freshwater and showed that their concentrations are low until one years old (r 0.23 and 0.8 ng/ml in both sexes, respectively) and also in two years old. But in age of 2.5 years old, in both sexes amount of E2, T and P were 3, 10.1 ng/ml and 7 pg/ml, respectively [4]. By comparison of this result with the present study, it was demonstrated that in brackish water, level of P was higher but that of T & E2 were lower than freshwater.

In study on immature white sturgeon clarified that in age 2 years old, level of T was lower than 1ng/ml in both sexes and this amount in males and females was 37.4 and 1.7ng/ml, respectively [8].

Investigation on mature and immature *Acipenser sturio* in brackish water proved that the highest amount of steroid hormones is T which in mature males and females was 40 and 20ng/ml, respectively. Amount of E2 & T in immature fishes was very little that it was probably resulted in less gonadic development. This research also showed a seasonal fluctuations of steroid hormone in immature fishes (more in spring) [9]. According to these results, seasonal fluctuations in steroid hormones weren't only in matured fish, but also could be observed in immature fish like present study.

In other research on Persian sturgeon, quantity of T in stage II was so little (0.25 ng/ml), in stage III increased (8.55 ng/ml) and in stage IV had a little decrease but remained at high level (7.44 ng/ml). E2 had a significant correlation with gonadic stage and in stage II was low (0.55 ng/ml) but in stage III (harmonizing with ovule growth) increased (4.53 ng/ml) and then in stage IV decreased (2.65 ng/ml). P also had a significant correlation with gonadic stage, so in stage II, III, IV was 0.32, 0.52, 0.36 ng/ml, respectively [7]. This report confirmed increasing of T & P in present study but the decrease of E2 is opposite of other reports and need more investigations about that.

Hormones in stage II, II-III of ovule development are low because in this stage (previtellogenic stage) ovule is small and animal and vegetable poles are not distinct. Results in present study were the same with other report about lower hormone in early stage of gonad development [9]. Stage of gathering vitellin in ovule (is still nucleus in center of ovule) affected by secretion gonadotropin hormone in pituitary gland, level of steroid hormone will be increased [10]. So, in present study production of steroid hormone in stage II-III also increased.

By comparing the present study with other similar studies it was ascertained that amount of T in both sexes in brackish water is lower, but amount of E2 is higher than other condition. Great sturgeon cultured in brackish water at stage II had higher amount of sexual hormone than other condition. In addition, both sexes of this species in brackish water have upper gonadic stage than other environments.

According to the results, it seems that sexual hormones fluctuate before maturity and affected by different seasonal and environmental condition. So, in order to investigate exact endocrine process and sexual steroid hormone production, these hormones should be measured before and in maturity stage.

Taking in to consideration overall results, hypothesis of being correlation between blood profile with gonadic stage of Great sturgeon cultured in brackish water was confirmed. The lower amount of steroid hormone in early stage, increase in until stage III and decrease in stage IV was proved. No relationship between P & E2 with gonadic stage indicated that in immature fish in upper stage (near maturity) all of steroid hormones affected by gonadic stage and had significant correlation in different gonadic stage.

Eventually it was clarified that culture medium of brackish water is more proper than fresh water and had a wonderful effect on gonadal growth and steroid hormones production progress in Great sturgeon and through sustainable management and use of effective factors on gonadic growth can be reduce long time of maturity in sturgeon cultured in brackish water and in order to caviar production in shorter time.

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