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THE STUDY OF SEASONAL STEROID HORMONES IN MALE SIBERIAN STURGEON (*Acipenser baerii*) FOR DETERMINING GONADAL DEVELOPMENT STAGES

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ABSTRACT

The aim of this study is to investigate of steroid hormones of male Siberian sturgeon in different seasons. For this, blood sampling was taken seasonally from 11 male Siberian sturgeons (7 years old) and then steroid hormones including testosterone and 11-keto testosterone were analyzed by using ELISA. The results showed that testosterone had no significant differences during all the seasons ($p>0.05$) and the maximum level was in autumn. But 11ketotestesterone had significant differences between winter with other seasons ($p<0.05$). The min and max level of 11ketotestesterone was in autumn and winter, respectively. 11-keto testosterone hormone levels based on sexual maturation stages showed significant difference between stages III and IV with stage II ($p<0.05$). Also, there was significant differences between stage II with others in testosterone levels ($p<0.05$).

Keywords: Siberian sturgeon, testosterone, 11keto testosterone, sex determination

INTRODUCTION

Sturgeons are the oldest chondrostean fish called alive fossils **(Baker *et al*, 2005).** Today, the population of these fish is decreasing because of overfishing, pollution, habitat degradation and dams **(Keyvanshokooh *et al*, 2010; Moghim et al., 2002; Flynn et al., 2006; Billard and Lecointre., 2000).** The family of Acipenseridae has economical values such as the expensive caviar production. So from the point of aquaculture, the rearing whole female population is more profitable than mix population **(Hassanzadeh Saber *et al*, 2008; Keyvanshokooh *et al*, 2010).** Among the species of this family, Siberian sturgeon (*Acipenser baeri*) is one of the species that has advantages including easy adaptation with culture condition, resident to environmental changes **(Pyka & Kolman, 2003),** high growth rate, low maturity age and fast caviar production **(Adamek *et al*., 2007).** So, to reach a successful management, it is important to know reproduction features **(Matsche *et al*, 2011).** *A. baerii* can reach a maximum length of 2 m and weight of 210 kg. However, it usually does not exceed 65 kg in weight with a maximum age of approximately 60 years. The spawning season is from May to June. *A. baerii* feeds predominantly on benthic organisms including chironomid larvae and river amphipods, isopods and polychaetes (**Sokolov and Vasil'ev, 1989**).
It is essential to know about sturgeons' reproduction cycle for optimal management of stocks and developing diagnostic methods of sex determination in low ages for aquaculture purposes **(Petochi *et al*, 2011).** Determining of reproduction status is difficult in sturgeons because they are sexually uniformic and don't have external dimorphism features and as well maturity age is late and have two or more reproduction cycle **(Barannikova *et al*, 2004).**
Reproduction in fishes regulates with a cascade of hormones along brain-pituitary- gonad called reproductive axis. Nervous gonadotropin releasing hormones directly affect pituitary gland and stimulate FSH and LH secretion. The most important steroid hormones are estradiol and 11-keto testosterone which play major roles in females and males, respectively. In males, 11-keto testosterone is the major regulator of spermatogenesis. FSH acts on sertoli cells and biosynthesises 11-keto testosterone by activating special enzymes like 11-β hydroxylase **(Cabrita et al., 2008).** Therefore, blood sex steroid hormones analysis is another gonadal development method which play important roles in gonadal development **(Scholz *et al*, 2009).** As mentioned above, the most important steroid hormones include testosterone (T), 11-keto testosterone (11-KT) and 17-β estradiol (E2). Plasma levels of these hormones in sturgeons are low before gonadal development stage and show considerable increase of testosterone and 11-keto testosterone levels by the cell division beginning in males. Also, in females and because of oocyte growth, testosterone and 17- β estradiol levels increase **(Barannikova *et al*, 2004; Davail- Cuisset *et al*, 2011).** Measuring of plasma sex steroid and glycophospholipoprotein levels has fewer stress compared with surgery and investigating of these hormones can be used in sex and gonadal development stages determination **(Craig *et al*, 2009).**

MATERIAL AND METHODS

Fish and culture condition

For this purpose, 23 individual of Siberian sturgeon (7 years) randomly selected in Shahid Dr. Beheshti Sturgeon Fish Propagation and Rearing Complex, tagged and transferred to fiberglass tanks (1× 1.8× 1.8 m^3) with 50 cm water depth. Water temperature was measured (16 C°).
First, fishes were anesthetized by clove powder (150 mg/lit) in water, then total length and weight measured by meter and scale, respectively, shown in table 1. Blood sampling was taken from caudal fin by using none heparinized syringe (5ml) and samples were centrifuged (4000 rpm in 10 min) and stored in -10 °C. ELISA procedure was used for male steroid hormones analysis (T & 11-KT, ng/ml) in endocrine gland research center in Tehran, Iran.

Data analysis

By general statistic and Excel, total length and weight mean and standard error were calculated. Independent samples t-test was performed to compare mean weight of females and males in each group and mann whitney test for T and 11-KT at 95% significance level, using SPSS 16.0 software. Data are presented as mean± standard error.

RESULT AND DISSCUSSION

The results showed that there were no significant differences in total length and weight of fish ($p> 0.05$). In table 1, total length and weight were given (Mean± SEM). The mean values of T and 11-KT are shown in table 2. Testosterone levels were 23±0.34, 22.3± 0.81, 23.5± 0.22 and 22.2± 0.58 (ng/ml) and 11-keto testosterone levels 1.35± 0.36, 1.38± 0.48, 1.01± 0.23 and 2.86± 0.4 (ng/ml) in spring, summer, autumn and winter, respectively. Testosterone hormone analysis demonstrated that there was no significant difference during all seasons ($p>0.05$)

and the highest level was in autumn. But in 11-keto testosterone, there was significant difference between winter with other seasons ($p<0.05$) and the highest and lowest levels were in winter and autumn, respectively (table.2).

The result of hormone analysis based on sexual maturity stages showed that testosterone had the highest and lowest levels in stages V and II, respectively (23.97 and 22.54 ng/ml, respectively). There was also significant difference between stage II with other stages ($p<0.05$). In other hand, the highest and lowest levels of 11-keto testosterone were in stages III and II (2.98 and 1.52 ng/ml, respectively). The results showed that there was significant difference between stages III and IV ($p<0.05$) (table.3).

Table 1 The mean of total length and weight of Siberian sturgeon 7 years old

Sex	No.	Total length (cm) (Mean ± SEM)	Total weight (kg) (mean± SEM)
female	12	100.57± 1.62	4.13± 0.18
male	11	94± 2.58	3.12± 0.34

Table 2 Steroid hormone levels in male Siberian sturgeon in different seasons

Hormone (ng/ml)	Spring (Mean±SEM)	Summer(Mean±SEM)	Autumn(mean±sem)	winter(mean±sem)
Testosterone(T)	23± 0.34 **a**	22.3± 0.81**a**	23.5± 0.22**a**	22.2± 0.58**a**
11-keto testosterone(11-KT)	1.35± 0.36**a**	1.38± 0.48**a**	1.01± 0.23**a**	2.86± 0.4**b**

Table 3 Steroid hormone levels in male Siberian sturgeon in different gonadal stages

Gonadal stage	Testosterone (Mean± SEM)	11-ketotestosterone (Mean± SEM)
II	22.54 ± 0.4**a**	1.52 ± 0.07**a**
III	23.6 ± 0.6**a**	2.98 ± 0.4**b**
IV	23.4 ± 0.2**a**	2.46 ± 0.3**b**
V	23.97± 0.1**a**	2.83 ± 0.1**b**

One of the gender and gonadal development stages determination is the measuring of steroid hormones such as testosterone, 11-keto testosterone and estradiol in wild sturgeons. In teleosts, dominant male androgens are testosterone and 11-keto testosterone that testosterone acts as a precursor of 11-keto testosterone and can participate in spermatogenesis process **(Aramli *et al*., 2013)**. Testosterone and 11-keto testosterone play roles in spermatogenesis and early spermatogenesis, respectively. Also, testosterone with enzymes such as 11-β hydroxylase and 11-β hydroxysteroid dehydrogenase converts to 11-keto testosterone. During spermatogenesis, androgen levels due to the decreasing conversion of 17β- hydroxyprogesteron to androgens and the change in the pathway of steroid to progestin formation decrease **(Barannikova *et al*., 2004)**.

In this research, 11-KT levels reach highest in winter and demonstrate an increasing trend in spring and summer, then a decreasing in autumn and finally increase in winter. Testosterone had a fluctuation trend during seasons which being increased in spring and autumn and decreased in summer and winter. Recently, plasma 11-KT level has been used for determining of immature Siberian sturgeons. According to **Cuisset *et al* (1991)**, 11-KT level used for immature Siberian sturgeon was 5 ng/ml, fish with 5ng/ml or more 11-KT classify as male; and fish with fewer than 5 ng/ml as female. If the threshold level decreased to 3ng/ml, immature females classification increases from 5% (4ng/ml) to 9% (3 ng/ml) for testosterone and 3% (4 ng/ml) to 7% (3 ng/ml) for 11-KT. Therefore, it seems that 4 ng/ml of testosterone or 11-KT can be used for immature females and males differentiation, but also should consider the error in immature males classification as female. However, in this paper 11-KT level was 2/86 ng/ml.

Sex steroids decrease after gonadal development near spawning and post spawning **(Craig *et al*., 2009; King *et al*., 1994; Rosenblum *et al*., 1987)**. In lake sturgeon, sex steroids increase in pre spawning and quickly decrease in post spawning **(McKinley *et al*., 1998)**. Similar results have been reported in other sturgeons about steroids and vitellogenin **(Amiri *et al*., 1996; Barannikova *et al*., 2004; Linarse-Casenave *et al*., 2003)**.

Despite of special sexual differences in productive hormone levels between male and female, these levels are not completely reliable just because of natural fluctuations during reproductive cycle. Vitellogenin produces in response to estradiol increasing during oocytes growth. Immature and males have much low or no vitellogenin. **Wildhabber *et al* (2007)** showed that with reproductive cycle existence, female vitellogenin level is 100 times higher than male. Since males mature in lower ages, and reproduce more than females, it is reasonable that more reproducing males than females exist during spawning cycle **(Craig *et al*., 2009)**. Although there is difference of steroid levels between species, plasma steroid profile is similar during sturgeon maturing and may be used for determining gender and maturing stages. Although there is error in classification of gender and maturing stages of white sturgeon by using plasma indexes, this method has advantages compared with biopsy **(Webb *et al*., 2002)**. According to **Bagheri *et al* (2008)**, the results showed that steroid hormone levels are influenced by gender, and testosterone in males and estradiol and progesterone in females were higher. Hormonal changes depend on environment temperature. The study on immature and mature *Acipenser sturio* in brackish water showed that the highest steroid hormones levels is testosterone. In immature fishes, estradiol and testosterone levels are so low that likely because of low gonadal development. Additionally, they showed seasonal fluctuation in immature fishes. Seasonal fluctuations of steroid hormones were seen not only in mature but also in immature fishes **(Davail- Cuisset *et al*, 2011)**.

Sex steroid hormone level measurements have little stress compared with surgery. Thus, hormone measuring can be used for determination of gender and gonadal development stage in the fishes **(Craig *et al*., 2009)**. In Persian sturgeon (*Acipenser persicus*) **(Viayeh *et al*., 2006)**, and shovelnose sturgeon (*Scaphirhynchus platorynchus*)**(Wildhaber *et al*., 2007**), these hormones were reported by more than 90% accuracy for sexual stage determination. Also, it is reported that testosterone level in male white sturgeons in stage II were higher than females **(Webb *et al*., 2002).** This property results in early sex determination in different gonadal development stages. **Semenkova *et al* (2006)** confirmed this result but showed that effective and reliable application of this method needs investigating of reproductive status and testosterone, 11-keto testosterone as well as estradiol in different ages of male and females in various farms(pond, circular and warm water). **Chebanov and Gallich (2009)** stated that one of the endocrine method disadvantage is the high expense in field and laboratory conditions. Blood analysis requires related equipments, fish tagging system and employees for capturing as well as analysis time. However, **Sakomoto *et al* (2001)** suggested that changes in blood parameters among fishes can be influenced by other variables including sampling, capturing, correct handling way, captive condition and analysis method.

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