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Determination of gender and sexual maturity stages of reared great sturgeon (*Huso huso*) using blood plasma sex steroid ratios

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Abstract

Gender and sexual maturity stages of great sturgeon (*Huso huso*) were studied using blood plasma sex steroids hormones ratios (17β-estradiol (E2), testosterone (T) and 11-ketotestostrone (11KT)}. One hundred sixty reared great sturgeon aged (1-12 years) with weight and length 0.45 to 49.65 kg and 36-198 cm, respectively. For each gender, 80 individuals (20 fish in each maturity stage) were selected out of 650 fish. Hormone concentrations measured with commercial kits. All samples analyzed in duplicate and a separate standard curve run for each ELISA plate. The findings of this study showed that it is possible to separate male and female fish especially from the second sex maturation stage using T/E2 and 11KT/E2 hormone ratios. The results showed that the use of E2/T, E2/11KT ratios are very useful indices for sex determination and sexual maturity stages assessment in male reared great sturgeon, but T/11KT and 11KT/T ratios were not functional. Based on the results, the use of T/E2 and 11KT/E2 ratios were preferable to E2/T and E2/11KT ratios for recognition of different sex maturation stages in male fish. These hormone ratios were used to determine different stages of male sexuality with more than 95% precision, and less than 30% for females. In addition, despite strong correlation between length and weight of fish with hormone ratios, sex could not identified through this parameter. The results of the present study revealed that some sex steroid hormones ratios can determine the sex and sex stages of reared great sturgeon especially in males with high accuracy.

Keywords: *Huso huso*, Gender, Sex maturity stages, Sex steroid hormones ratios

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Introduction

Gender determination and sex staging in reared fish species is one of the most important prerequisites for brood stock. Individual identification of the spawned fish sex is inevitable in order to maintain the desired sex ratio, production of suitable fingerling for aquaculture and the restoration of natural reserves (Chu-Koo *et al.,* 2009). External signs can easily identify adult of male and female of many fish species. However, some species do not have any external signs or marks for gender identification even at the stage of final sexual maturity. Sturgeon including *Huso huso,* are species with lack of external signs of sex despite of their maturity age and long reproductive cycle (Holcik, 1989) and the absence of sexual dimorphism even after maturity (Doroshov *et al.,* 1983). Therefore, sexing of sturgeon is always a challenge. Improving the methods of sex determination in this group of fish is an unavoidable need for modern breeding management. Because traditional methods of sex determination, such as biopsy and surgery, have clear and latent effects like physical and physiological injury to gonad, delay or absence of ovulation and mortality (Du *et al.,* 2017), could not be economical and appropriate methods.

Great sturgeon, the largest fish in brackish water, is one of the most important species of farm sturgeon in the world and an endemic species to the Caspian, Azov and Black seas (Falahatkar and Poursaeid, 2014). This species as well as other endangered species of sturgeon (IUCN, 2009), requires cultured brood stock. Meat and caviar production are two major goals of *H. huso* breeders. It takes a long time (at least 8 years) to get caviar, but it takes less than 4 years to produce meat. The average of age at maturity for great sturgeon females and males in nature and reared conditions are 18, 15 and 9, 4 years, respectively. Therefore, to manage the costs of rearing and to make its production economy, it is necessary to separate the male and female for production of meat and caviar.

The concentration of sex steroid hormones and other biochemical parameters of blood plasma as an aggressive techniques have been reported to sex determination of sturgeon (Webb *et al.,* 2002; Barannikova *et al.,* 2004; Malekzadeh Viayeh *et al.,* 2006; Wildhaber *et al.,* 2007; Craig *et al.,* 2009; Esmaeili Mola *et al.,* 2011; Falahatkar and Poursaeid, 2014; Kazemi *et al.,* 2014; 2019; Du *et al.,* 2017) and bony fishes In recent years (Takemura and Oka, 1998; Pottinger and Carrick 2001; Chu-koo *et al.,* 2009). These techniques are less expensive and easier than traditional methods of sex determination (biopsy and surgery). However previous studies have been shown that the testosterone (T), 11-ketotestosterone (11KT) and 17β-estradiol (E2) can determine the difference between male and female when the gonads are well-developed and in high maturation stages (Du *et al.,* 2017). In fact, the direct use of sex steroid hormones to determine the sex of young fish cannot be accurate.

In general, in male fish the concentration of androgens hormones is higher than the concentration of estrogens hormones and vice versa. The ratio of estradiol to androgenic hormones (E2 to T and E2 to 11KT) are expected to be better indicators than the individual concentration of sex

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steroids for sex determination. Because, these ratios indicate differences between males and females notwithstanding of the absolute concentration of steroid hormones (Snyder, *et al.*, 2004). In addition, some studies in teleost fishes showed that the change in the ratio of E2 to T in female was greater than that of T to E2 alone (Giesy *et al.*, 2003).

Therefore, the use of sex hormones individually for sex determination and sex staging of sturgeon is limited. However, the use of hormone ratios can improve this problem to some extent. The aim of this study was to attainment a more accurate diagnosis of sex determination and sexual maturity stages of reared great sturgeon based on different ratios of sex steroid hormones of T, 11KT and E2.

Material and methods

Fish

This study carried out in Physiology and Biochemistry and Aquaculture Departments of the International Sturgeon Research Institute (ISRI), Rasht, Iran during 2014 to 2017. One hundred and sixty great sturgeons (*H. huso*) ranging from 1-12 years of age, 0.45-4.95 kg of weight and 36-198 cm of length randomly selected from 650 fish. The reason to use quite wide age classes in this research was to ensure the occurrence of a specific sexual stage II, III, IV), and (I in the first reproductive cycle of fish.

For each of 4 sexual maturity stages 20 males and 20 female fish (overall, 80 females, and 80 males) selected using laparoscopic images. In the present study, all fish have been artificially propagated at ISRI from wild brood stock caught from the Caspian Sea (F1) and farmed beluga (F2). These fish reared in earthen and concrete ponds under natural photoperiod with water temperatures between 5 to 10°C in winter and 30 to 12°C during other seasons. The fish were fed with a commercial sturgeon formulated diet (containing 41% protein, 12% fat and 3% cellulose, Faradaneh Company, Iran), at 0.5 to 3% of body weight depending on season, size and environmental conditions.

Sex determination and maturity staging of these analyses were determined by measuring blood plasma steroid hormones concentrations. Prior to examination, fish starved for at least 24 h and then anesthetized in a tank containing (700 mg L^{-1}) clove powder (*Caryophillium aromaticus*) for 6-8 minutes. Then, the fish placed on a table for examination and biometric data (total length and weight). The age of experimental fish obtained from propagation and rearing department of the ISRI.

Sex steroid hormones

Blood samples collected immediately from the caudal vein of each fish using a heparinized syringe. For each fish up to 3-ml of blood was taken to measure the plasma steroid hormone levels including 11KT, T and E2. From each individual, a 2-ml blood collected in blood collection tubes and transferred to the Physiology and biochemistry Department for hormone analysis. Immediately after sampling, the blood samples centrifuged at 3000 rpm for 10 minutes plasma samples were aliquot and stored at - 80°C until further analysis. Hormone concentrations were measured with commercial kits (11KT: East Biopharm Hangzhou China with CK-E90827 and CK production code) and E2

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and T (Monobind US company, with production code of 4925-300 and 3725-300 respectively). All samples analyzed in duplicate and a separate standard curve run for each ELISA plate. Measurements were done at 450 nm wavelength using a Biotek ELX800 plate reader (Semenkova *et al.*, 2002). Then, based on the concentration of sex steroid hormones ratios obtained the hormone ratios T/E2, 11KT/E2, E2/T, E2/11KT, T/11KT, and 11KT/T calculated separately for each group of males and females fish in four different stages of sexual maturity.

Histology

Biopsies of gonads collected using laparoscopic sampling forceps from posterior a single gonad. Gonad tissues fixed in Bouin's solution for 48 h and stored in 70% ethanol until histological processing. Gonad tissues were processed routinely by dehydration in a graded series of ethanol solutions, followed by clearing in a series of xylenes, embedded in paraffin, and sectioned at 7-µm. Prepared slides were stained with hematoxylin and eosin (H&E) and studied by light microscope (Nikon E600, Japan). Three slides were prepared for each sample. The sex and maturity stage for each fish assessed according to the protocol defined by Flynn and Benfey (2007).

Statistical analyses

The data of hormonal indices in different maturity stages were analyzed (for each sex separately) using one-way ANOVA and the difference between the meanings was determined with Duncan's multiple comparison. Normalization of data was examined using Kolmogorov– the Smirnov test. A comparison was made between the sex hormones concentrations male and female using independent t-test. An independent t-test used to compare the sex hormones concentrations of both sex at different maturity stages. The tests were performed in SPSS software (version 20) at error level (p<0.5). All data presented in the text are mean and standard error (Mean±SE).

Results

The results indicated a significant difference in all of the sex hormone ratios between male and female at the first stage of maturity (t=15.38, 7.01, 418.31, 16.60, 176.86 and 282.10, respectively, p<0.05 and df =38) (Table 1). Nevertheless, in the second stage of sexual maturity with the exception of the 11KT/T ratio (t=2.49 with p>0.05 and df =38), the other hormone ratios were significantly different (t=13.14, 15.55, 26.37, 15.26 and 5.20 respectively, with p<0.05 and df=38). In the third sex maturity stage, the T/11KT ratio (t=0.16 with p>0.05 and df=38) was not significantly different, but the other hormone ratios were significantly different in both males and females (t=32.54, 10.53, 40.56, 9.77 and 9.49 respectively with p<0.05 and df=38). In the fourth stage, T/11KT (t=0.13, p>0.05, df=38) and 11KT/T ratios (t=0.11, p>0.05, df=38) were not significantly different, but the other sex hormone ratios studied varied significantly different (t=9.53, 11.48, 6.31 and 8.88 with p<0.05 and df=38, respectively).

The findings of this study showed that the mean of E2/T and E2/11KT ratios in both sexes were less than 1 in all stages of sexual maturation (except E2/T ratio for

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the first stage of female which was more than 1) and in females always was higher than males (Table 1). In addition, the mean of 11KT/E2 and T/E2 ratios in males and females were more than 1in all sex maturity stages. These ratios in males were much higher than females in all sex stages. the mean of E2/11KT and E2/T ratios of female to male at the first to fourth stages of sexual maturity were 2.19, 3.64, 5.45 and 4.67 times and 7.38, 4.10, 8.33, and 4 fold respectively. In addition, the mean of 11KT/E2 and T/E2 ratios in male to female at the first to fourth sex maturation stages were 2.18, 3.60, 5.21 and 5.86 times and, 3.40, 4.02 and 3.40 fold respectively (Table 1).

Table 1: Mean±standard error values of hormone ratios at different stages of sexual maturity in reared *Huso huso* (n=20 for each sex in each maturity stage).

Hormone ratios	sex	Sex stage I	Sex stage II	Sex stage III	Sex stage IV
E2/11KT	male	0.41±0.01	0.22±0.01	0.11±0.00	0.03±0.01
	female	0.90±0.04	0.80±0.02	0.60±0.01	0.14±0.01
female/male (fold)		2.19	3.64	5.45	4.67
male/female (fold)		0.45	0.27	0.18	0.21
11KT/E2	male	2.51±0.08	4.56±0.13	8.86±0.23	44.33±2.60
	female	1.15±0.05	1.27±0.03	1.70±0.04	7.56±0.42
female/male (fold)		0.46	0.27	0.19	0.17
male/female (fold)		2.18	3.60	5.21	5.86
E2/T	male	0.21±0.01	0.1±0.00	0.06±0.00	0.03±0.01
	female	1.55±0.26	0.41±0.01	0.50±0.01	0.12±0.01
female/male (fold)		7.38	4.10	8.33	4.00
male/female (fold)		0.13	0.24	0.12	0.25
T/E2	male	4.77±0.17	10.04±0.24	16.00±0.34	52.39±3.14
	female	1.40±0.24	2.50±0.30	2.02±0.06	8.72±0.43
female/male (fold)		0.29	0.24	0.12	0.16
male/female (fold)		3.40	4.02	7.92	6.01
11KT/T	male	0.53±0.01	0.46±0.02	0.55±0.01	0.85±0.01
	female	1.62±0.26	0.51±0.01	0.85±0.02	0.88±0.04
female/male (fold)		3.06	1.11	1.54	1.03
male/female (fold)		0.32	0.90	0.64	0.96
T/11KT	male	1.90±0.03	2.23±0.08	1.81±0.04	1.18±0.01
	female	1.13±0.18	1.98±0.05	1.20±0.03	1.17±0.05
female/male (fold)		0.59	0.88	0.66	0.99
male/female (fold)		1.68	1.13	1.51	1.01

The results showed that the efficiency of T/E2 and 11KT/E2 sex ratios for separation of great sturgeon males and females were higher and easier than E2/T and E2/11KT sex ratios (Figs. 1 and 2). In addition, the 11KT/T and T/11KT ratios lacked the ability to sex identification and maturity staging in both males and females (Table 3). Based on the results, the accuracy of sex staging using hormone ratios of E2/T, T/E2, E2/11KT and 11KT/E2 was >95% and <25% for male and female, respectively (Table 2).

These results showed that the correlation between length and weight of reared great sturgeon (male and female) with different sex hormone ratios was not the same. The correlation of fish length and weight was very strong with E2/11KT and E2/T ratios and with 11KT/E2 and T/E2 ratios but weak with 11KT/T and T/11KT ratios (Table 3).

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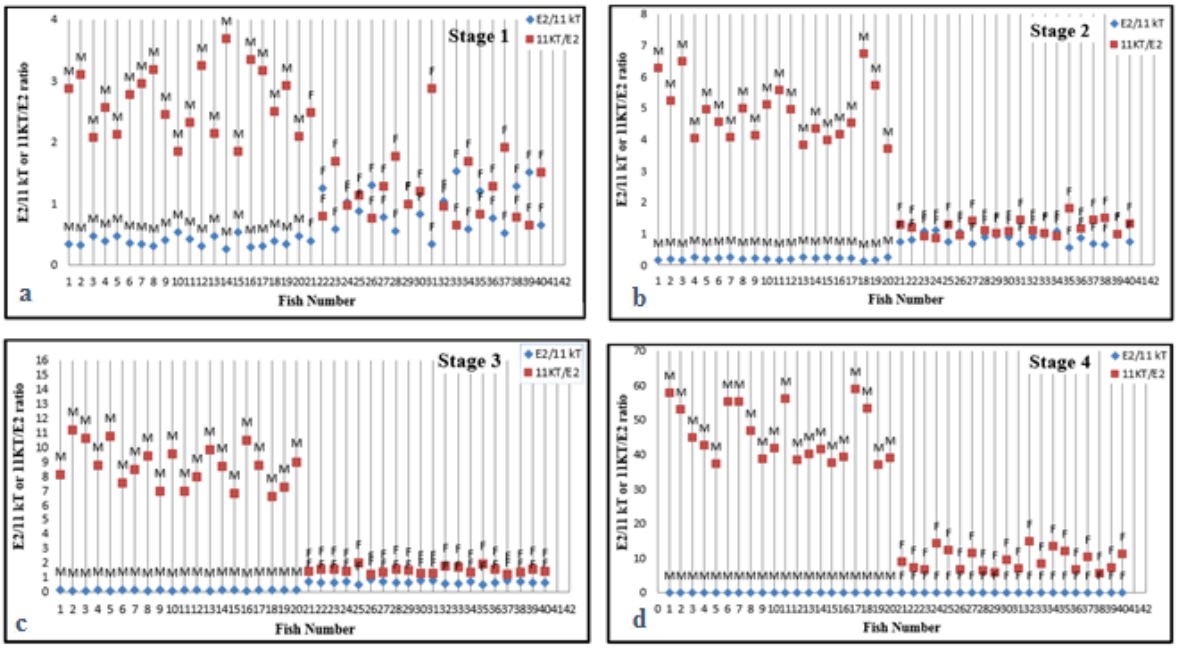

Figure 1: Sexing the farmed great sturgeon (*Huso huso*) using E2/11KT and 11KT/E2 ratios in sex maturation stages. Stage I (a); stage II (b); stage III (c) and stage IV (d).

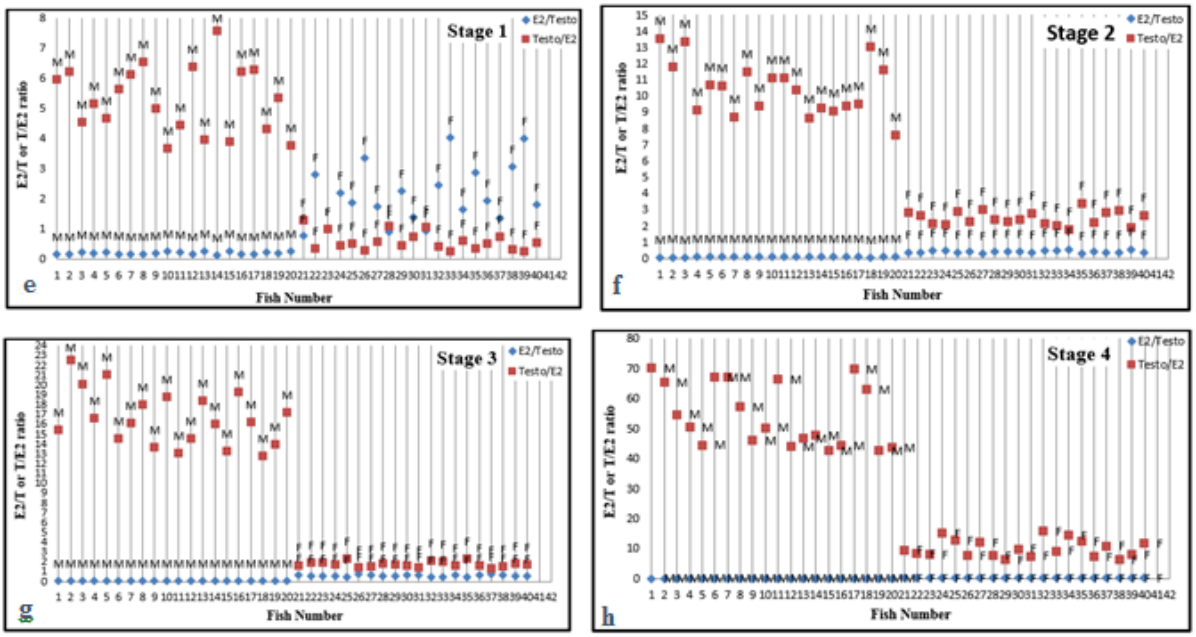

Figure 2: Sexing the farmed great sturgeon (*Huso huso*) using E2/T and T/E2 ratios in sex maturation stages. Stage I (e); stage II (f); stage III (g) and stage IV (h).

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Table 2: The range of individual sex steroid hormones ratios in reared *Huso huso* at different sex maturation stages (n=20 for each sex in each stage).

Hormone ratios	sex	Sex stages			
		I	II	III	IV
E2/T	male	0.16-0.17	0.08-0.11	0.05-0.07	0.01-0.02
	female	0.38-3.10	0.31-0.50	0.42-0.63	0.07-0.10
T/E2	male	3.66-6.14	8.65-12.68	13.18-20.56	43.86-67.73
	female	0.32-2.71	1.98-3.17	1.56-2.37	5.35-10.86
E2/11KT	male	0.32-0.57	0.17-0.29	0.08-0.13	0.02-0.03
	female	0.71-1.35	0.58-0.96	0.51-0.72	0.09-0.22
11KT/E2	male	1.74-3.16	3.40-5.82	6.84-11.86	36.80-56.93
	female	0.74-1.37	1.04-1.71	1.39-1.95	4.45-11.37
11KT/T	male	0.48-0.63	0.35-0.67	0.42-61	0.80-0.95
	female	0.42-3.42	0.42-0.63	0.70-1.11	0.56-1.50
T/11KT	male	1.66-2.10	1.74-2.82	1.63-2.40	1.05-1.26
	female	0.30-1.99	1.59-2.40	0.90-1.48	0.66-1.80

Table 3: Pearson correlation coefficient between sex ratios, length and weight of male and female reared *Huso huso* (n=80 for each sex).

Hormone ratios	sex	Hormone ratios							
		Length	Weight	E2/11KT	11KT/E2	E2/T	T/E2	11KT/T	T/11KT
Length	male	1	0.965**	-0.916**	0.622**	-0.908**	0.672**	0.560**	-0.517**
	female	1	0.957**	-0.646**	0.544**	-0.738**	0.610**	-0.327**	-0.106
Weight	male	0.965**	1	-0.875**	0.696**	-0847**	0.733**	0.678**	-0.650**
	female	0.957**	1	-0.730**	0.670**	-0.630**	0.693**	-0.220	-0242*
E2/11KT	male	-0.916**	-0.875**	1	-0.727**	0.992**	-0.773**	-0.609**	0.515**
	female	-0.646**	-0.730**	1	-0.814**	0.708**	-0.801**	0.027	0.277*
11KT/E2	male	0.622**	0.696**	-0.727**	1	-0.694**	0.994**	0.901**	-0.793**
	female	0.544**	0.670**	-0.814**	1	-0.449**	0.982**	-0.036	-0.239*
E2/T	male	-0.908**	-0.847**	0.992**	-0.694**	1	-0.745**	-0.558**	0.463**
	female	-0.738**	-0.630**	0.708**	-0.449**	1	-0.546**	0.270*	-0.064
T/E2	male	0.672**	0.733**	-0.773**	0.994**	-0.745**	1	-0.880**	-0.773**
	female	0.610**	0.693**	-0.801**	0.982**	-0.546**	1	-0.128	-0.141
11KT/T	male	0.560**	0.678**	-0.609**	0.901**	-0.558**	0.880**	1	-0.967**
	female	-0.327**	-0.220	0.027	-0.036	-0.270*	-0.128	1	-0.837**
T/11KT	male	-0.517**	-0.650**	0.519**	-0.793**	0.463**	-0.773**	-0.967**	1
	female	-0.106	-0.242*	0.277*	-0.239*	-0.064	-0.141	-0.837**	1

** Different letters in each row means there is a significant difference (p<0.01).
* Different letters in each row means there is a significant difference (p<0.05).

Histology

Gonad tissues at maturity stage I to stage IV males (Figs. 3a, c, e, and g) and females (Figs. 3b, d, f, and h) were distinguished according to differences in color, shape of cell, fat content, size, and structure. We used histology for confirming the accuracy of used method.

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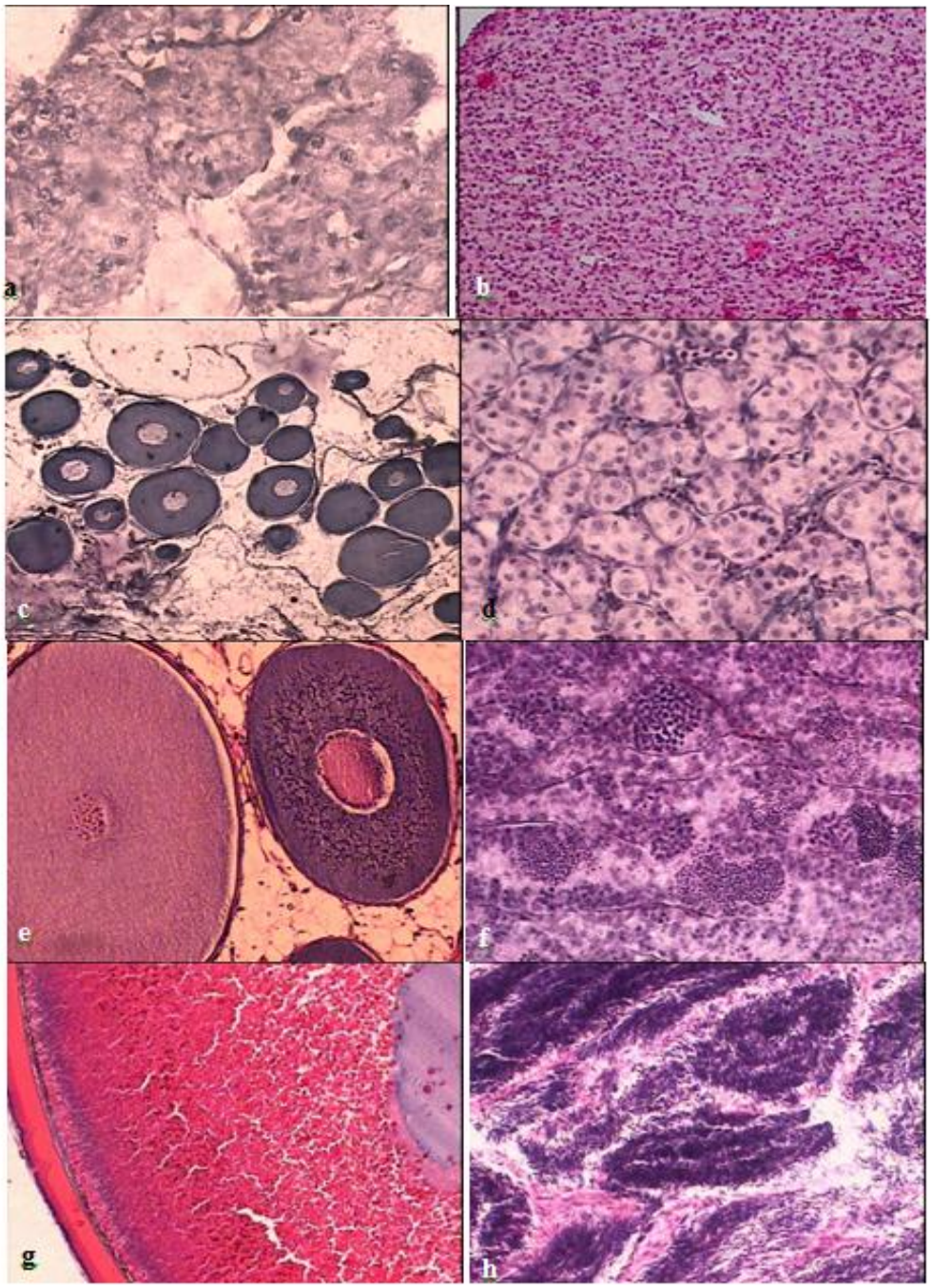

Figure 3: Histological section and maturity stage of ovary and testis of cultured *Huso huso* at the different sex stages; ovary: stage I (a|), (stage II (c), stage III (e) and stage IV (g); testis: stage I (b), stage II (d), stage III (f) and stage IV (h). Hematoxylin-Eosin staining and magnification of objective lens: c=4x; b & e=10x; a, d, f, g &, h=20x).

Discussion

Sex identification of sturgeons, including great sturgeon were done, through different methods such as ultrasound (Du *et al.,* 2017), morphometric (Falahatkar and Poursaeid, 2014), histology (Bahmani and Kazemi, 1998; Chapman and Park, 2005), Laparoscopy (Falahatkar *et al.,* 2011; Kazemi *et al.,* 2014; 2019), sex steroid hormones (Webb *et al.,* 2002; Kazemi *et al.,* 2019). Despite many studies mentioned, there is still no appropriate and accurate method to determine sturgeon sex. Some researchers have suggested that

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concentrations of the hormones E2, T and 11KT alone may not be suitable indicators for sex identification. Nevertheless, sex ratios of E2 to T and E2 to 11KT allow separation of males and females (Ceapa *et al.,* 2002). In the present study, it was found that in cultured female great sturgeon, altering the T/E2 and 11KT/E2 ratios were more effective and efficient than the concentration of T, E2, and 11KT hormones alone. Although, 11KT and T are the dominant sex steroid hormones in male fish (Barry *et al.,* 1990) and 11KT is the major androgenic hormones of male sturgeon (Kazemi *et al.,* 2019) and carp (Koldras *et al.,* 1990), these two hormones play some important roles in female (Kazemi *et al.,* 2019). Testosterone, which is a male hormone, can act as an E2 precursor hormone in the ovary (Mc Master *et al.,* 1995), therefore, the strong effect of T concentration on E2/T ratio is not impossible (Giesy *et al.,* 2003). These results are consistent with the results of a study on common carp (*Cyprinus carpio*) suggesting that alteration of E2/T ratio in female was more effective than the individual hormone of T and E2 (Goodbred *et al.,* 1997).

The findings of this study showed that the E2/T ratio in both sexes was always less than 1 in all stages of sexual maturation (except for the first stage of sexual maturity which was more than 1) and in females always was higher than males. The range of E2/T ratio in the males and females of reared *H. huso* in this study at different stages of sexual maturity were 0.03-0.21 and 0.12-1.55, respectively (these ratios in females were at least 4 times more than males). It is possible the accuracy of this ratio be various for sex determining at different sex maturity stages in males (Table 3). The accuracy of sex determination at different maturity stages was less than 25% for females by using this ratio. Similar studies in common river carp showed that the E2/T hormone ratio in females was greater than males and always greater than 1 (Goodbred *et al.,* 1997). Although this ratio in females golden carp (*Carassius auratus*), was reported to be 2.5 times higher than males, the ratio in both gender was less than 1 (Giesy *et al.,* 2003). In the study on common carp, it was also found that the E2/T ratio of male and female was in the range of 0.15-1.77 and 0.17-3.86, respectively (Snyder *et al.,* 2004). Therefore, the results of this study were concordance with the findings of the above researches. This conclusion that the E2/T ratio in female fish is even greater than 1 cannot supported by general confirmation, because, based on the research results of the above-mentioned researches, even in two different studies on the same species (common carp), this hypothesis was not proven. It seems that the recrudescence of gonadal stages and differences in the geography location of strains and species appear changes in sex steroid profiles and E2/T ratio in relation to the reproductive cycle (Snyder *et al.,* 2004). As various environmental factors such as water temperature can affect the ovary of the pre-vitellogenesis, the conversion of T to E2 (Mc Master *et al.,* 1995) and the genetic developmental process of gonads will be affected.

Therefore, the plasma E2 concentration in the vitellogenesis stage and T concentration increased during most of the sexual maturation and post- vitellogenesis

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stage (Manning and Kime 1984; Kazemi *et al.,* 2014; 2019). Since, the activities of the aromatase enzyme decrease in the post-vitellogenesis stage (Colombo *et al.,* 1982; Zhang *et al.,* 2011). For this reason, larger values of E2 to T ratio especially in the male (even if there is no significant difference) may indicate lower levels of sexual maturation and vice versa.

The results showed that the T/E2 ratio as well as the E2/T ratio could be used to determine the sexing and sex staging of sturgeon. Although the mean values of T/E2 ratios in both males (4.52-77.39) and females (1.40-8.72) were greater than 1, these values in male was 3 to 8 times more than female at different stages of sexual maturity. The results of T/E2 ratio indicate the possibility of separation of male and female-farmed great sturgeon from the second stage of sexual maturity. The results of this research showed that the use of T/E2 ratios was more effective and more functional than E2/T ratios. As various values of T/E2, ratios were significantly different for determination of sexual maturation stages in male cultured *H. huso*. In addition, higher values of this ratio indicated higher levels of male sexual maturity. So that, having the range of these values individually, the sex stage of the males defined with more than 95% accuracy (Table 3). Nevertheless, it is very important to pay attention that accuracy of female great sturgeon sex determination using T/E2 ratio cannot be over 30%. In addition, no studies have been performed to sex determination and maturity stages of sturgeon and bony fish using T/E2 ratio of blood plasma.

Some studies shown that blood cells, adrenal glands, and ovaries have enzymes that can produce the 11KT from some other sex hormones to use in the process of gametogenesis and final sexual maturation of fish. (Cuiset *et al.,* 1995; Ceapa *et al.,* 2002). Previous studies on some sturgeon species shown that blood plasma 11KT and T are closely related to each other, so their concentration increases with increasing maturity (Barannikova *et al.,* 2004; Aramli *et al.,* 2013). As T is also a precursor hormone of 11KT. Hence, any changes in blood plasma 11KT concentration can affect on values of E2/11KT and 11KT/E2 ratios. The blood plasma E2/11KT ratio studied values of all male and female *H. huso* were in the range of 0.03 to 0.41 and 0.14 to 0.90, respectively. The mean E2/11KT ratio for females at each sex stage was more than 2-5.5 times that of males. Therefore, a ratio greater than one, E2/11KT in all female, indicated their normal status in this study. Similar results also found in another study on common carp (Snyder *et al*., 2004). They found that the blood plasma E2/11KT ratio values of male carp ranged from 0.14 to 1.13 (in only one case) and were approximately less than 1. Nevertheless, the range of this ratio, as in the present study, was higher in females (0.17 to 5.16) and, on average, 2-9 times of males.

The results of this study showed that the 11KT/E2 ratio values could use from the second stage of sexual maturity with more than 95% accuracy to separate male and female. Because, unlike E2/11KT ratios that were not able to separate at different maturation stages in both sex (in particular the female), but this ratio was able to separate at different maturation stages of male with significant difference. The

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efficiency and accuracy of the 11KT/E2 ratio to sex determination and staging of the great sturgeon were approximately similar to the T/E2 ratio. The very strong and positive correlation between these two hormones (T and 11KT) and efficient role of them in sexual maturation (Ceapa *et al.*, 2002) and sex determination of farmed great sturgeon (Kazemi *et al.*, 2019) could be the reasons for the similar performance. Studies on the use of 11KT/E2 ratios to sex determination of sturgeon are very limited (Ceapa *et al.*, 2002). The results of a few carp studies also showed that the numerical values of the hormone ratios in all cases were higher than the numerical values of the results obtained in this study (Goodbred *et al.*, 1997; Giesy *et al.*, 2003; Snyder *et al* 2004). It seems that the difference in the comparative amount of hormonal ratios may be species-dependent. In this study, individual results of 11KT/T and T/11KT ratios in both sex of male and female showed that these hormonal ratios are not suitable for sex identification and gonad staging. As in both sexes the levels of 11KT increased or decreased, respectively, with increasing or decreasing levels of the T hormone (T levels were always higher than 11KT levels at all sex maturation stages). Because of the increasing or decreasing trend of these two steroid hormones in the developmental process of gonads, so the ratios of these two hormones were not significantly different even at the same sex stages. As a result, the ratios of these two hormones were not significantly different even at the same sex stages.

The results of this research showed that the correlation between length and weight of fish (male and female) with sex hormone ratios were not the same. So that the correlation of fish length and weight was very strong with E2/11KT and E2/T ratios, strong with 11KT/E2 and T/E2 ratios and weak with 11KT/T and T/11KT ratios. The correlation between length and weight of males was higher than females in all hormone ratios. Although the results of this correlation (correlation between length and weight with hormone ratios) somewhat confirm sex identification and sex maturity stages, but these data cannot directly be used in sexing and sex staging. Thus, the correlation between the ratios of steroid hormones and the length and weight of the reared great sturgeon was observed similar to carp (Giesy *et al.*, 2003). This study showed that some sex steroid hormones ratios such as E2/11KT, E2/T, T/E2 and 11KT/E2 have high accuracy in determination of sex in reared great sturgeon, but the use of T/E2 and 11KT/E2 ratios was preferred over the E2/11KT and E2/T ratios. These hormone ratios were used to determine the different stages of male sexuality with more than 95% precision, but less than 30% for females. In contrast to the above hormone ratios, the T/11KT and 11KT/T ratios lack the necessary functionality to sex determination and sexual maturation. In addition, despite high correlation between length and weight of fish with hormone ratios, but sex identification was not possible with this parameter. The results of the present study provide useful basic information for future studies to use this method in commercial farming of sturgeon and management of wild stocks propagation.

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