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ORIGINAL RESEARCH

Study of steroid hormones, biochemical parameters and body size based on different gonad's quality and gonad's fat content from sonogrphy method in female cultured Siberian sturgeon (*Acipenser baerii*)

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Abstract Farmed female Siberian sturgeon's gonad (n= 30, 3-year old) was examined using an ultrasonograph unit with a 9–13 MHz linear transducer and results were compared with serum steroid hormones, biochemical parameters and body size. The sonogram was practically interpreted based on two indices as gonad's size based on gonad's size and gonadal fat content in the three classifications (small, medium, and large). As evidenced, 73.3% of fishes were considered as small gonadal fat content; adversely most of which had large gonad's quality. From the results, gonad's quality showed significant correlation with body size, in which larger fishes had medium and large gonad's quality. This ensures the more success of production of females for both caviar production and broodstocks production purposes when larger fishes in the same age are known in a farm and could be separated for better management. Pearson's correlation showed that there was a significant positive relationship between body length and triglyceride ($R = 0.571$, $P < 0.01$) and between body weight and triglyceride ($R = 0.665$, $P < 0.000$). Furthermore, a positive trend of triglyceride level in large gonad's quality group rather than small group could certify it as a potential serum's biomarker to detect larger female accompanied with relatively larger and more productive gonad in Siberian sturgeon.

Keywords Siberian sturgeon . Gonad's quality . Gonadal fat content . Ultrasonography . Serum steroid hormones

Introduction

Production of sturgeon for both meat and caviar will increasingly have to rely on aquaculture (Logan et al. 1995). In sturgeon aquaculture, it is important that sex of fish be identified early in development so that males can be reserved for meat production and then they are easily harvested at 2–4 years of age. Females used for caviar production (until 5–9 years of age) or as broodstocks (Bronzi et al. 2011).

Recognition of gonadal feature in sturgeon is very important for caviar production, early maturity and recruitment in this species that is under extinction (Hedayati and Bagheri 2010). Measurement of reproductive hormones concentration (e.g. estrogen, progesterone, and testosterone) or other metabolic indicators like the yolk precursor vitellogenin are possible methods to study the gonadal feature (Chapman and Van Eenennaam 2009). Webb et al. (2002) identified immature and maturing males and females of white sturgeon (*Acipenser transmontanus*) using plasma testosterone levels. Feist et al. (2004) successfully determined the sex of young cultured white sturgeon using plasma testosterone. In larger female Persian sturgeon (*Acipenser persicus*), serum testosterone and 17β-estradiol levels showed significant relationship with larger fish sizes (Nazari and

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Ghomi 2010). However, for determining the sex using this method samples take at least a day to analyze in a laboratory, and currently no portable or field tests are available (Chapman and Van Eenennaam 2009).

In commercial sturgeon farms, there has been an interest in finding noninvasive and fast techniques for determining sturgeon sex (Wildhaber et al. 2005; Chebanov and Chmyr 2005). The ultrasound method is noninvasive, as it allows determining the gender and gonad stage of fish without any additional stress other than handling (Chebanov and Galich 2009). Memis et al. (2016) investigated sonographic sex identification of Russian sturgeon (*Acipenser gueldenstaedtii*) and a lot more studies performed to determine the sex in other species. Ultrasound imaging has been shown to be effective to determine the sex of the 3 years old great sturgeon (*Huso huso*) with overall accuracy of 97.5% (Masoudifard et al. 2011).

Management strategies for farmed sturgeon species depend on knowing echogenicity of gonad of individual fish, being able to take further strategies for feeding and sex identification (Chapman and Van Eenennaam 2009). Although ultrasound is not known as a new method in the study of gonadal status, but during sex determination and female selection at stage II of maturity, different rate of sonogram images can be seen in female sturgeon so that gonad images can be observed either as hypereco (intense color) or hypoeco (weak color)(Vajhi et al. 2011). In some female, with hypoechoic (darker) gonad's images, gonad may be wrongly regarded as the male gonad. Therefore, sex identification using only ultrasonography is not a full reliable method of sex determination in sturgeons and needs a professional skill of sonographer to be certified in order, two qualitative tools in sonogram including gonad's size and gonadal fat content also regarded a new approach to determine the quality of gonad that again requires the higher professional skills of sonographer.

There might be a relationship between gonad's quality and gonadal fat taken from sonogram images and sex steroid hormones, biochemical parameters and body size that may help to increase the accuracy of sex identification in sturgeons. However, thus far such information is not much available in-deep, this study aimed to investigate any possible differentiation in Siberian sturgeon gonad's quality and gonad's fat content in three classifications in each associating to the body size. Results were also compared with serum hormonal and biochemical parameters.

Materials and methods

Fish samples

Fish samples (female 3-year-old; 4963.33 ± 1594.49 g weight; 93.56 ± 9.61 cm total length; n = 30) obtained form Saee Sturgeons Aquaculture Center (Sari, Iran) were raised in concrete tanks (8 m diameter × 1.5 m depth), with water temperature of 18±2 ºC, oxygen level more than 7 mg/l, and pH=7.6 ±0.02 throughout the year. All fish already being sex determined and separated as female in this farm by using sonography. The fish were transferred to a fiber glass tank and were anesthetized with clove powder at the concentration of 150 ppm for 5-10 min (Hallajian et al. 2007). After anesthetization, fish were placed on a canvas stretcher to prevent injury during gonad inspection.

Ultrasonography (USG)

Siberian sturgeon gonads were imaged using a portable ultrasound device (SonoAce R3, Medison) according to Masoudifard et al. (2011). Linear transducers with frequencies ranging from 9 to 13 MHz were used to increases the resolution of the image at early ages of the fish (Chebanov and Chmyr 2005; Wildhaber et al. 2005). To inspect gonads, the transducer was placed on the left and right sides of the fish's abdomen where the gonad is likely supposed to be and completely scanned to produce an image in partial transverse section. This location provided reliable images with easily identifiable land mark organs. The sonogram images for the size of the gonads (small, medium and large) and surrounding gonadal fat content (small, medium and large) were taken precisely to differentiate the ovary for different specimens.

Measurement of serum sex steroid hormones

From the caudal vein of anesthetized fish, 4-5 mL of blood samples were taken using a 5 mL plastic syringe

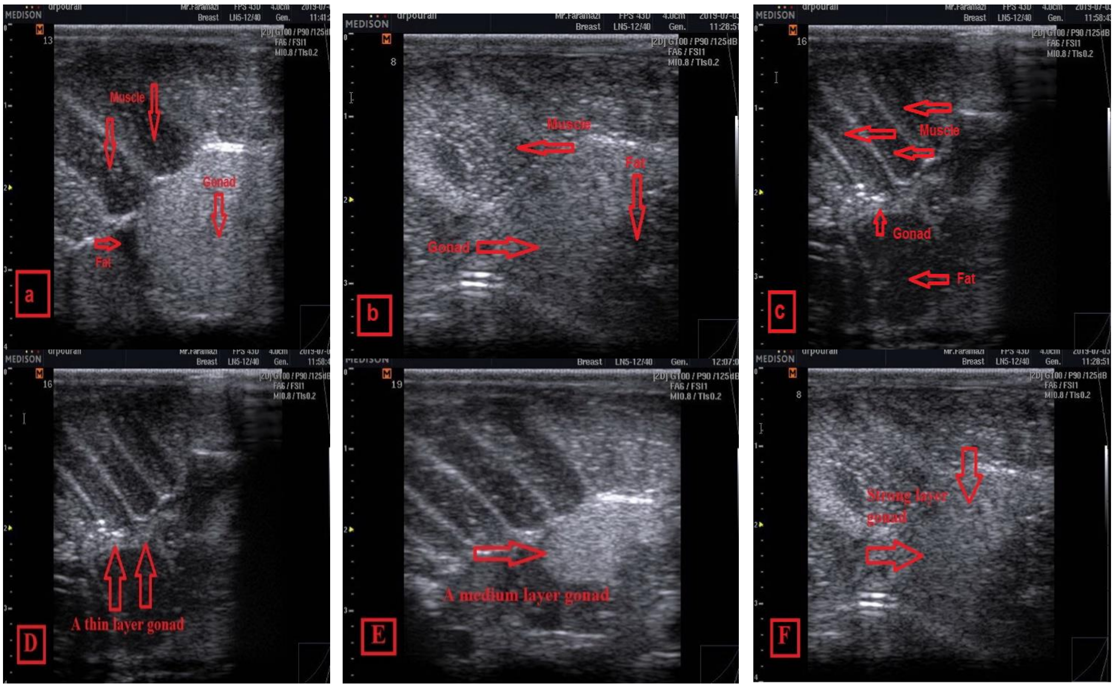

Fig. 1 Transverse sonogram of 3-year old female Siberian sturgeon; (a) low fat ovary, (b) medium fat ovary, (c) large fat ovary, (D) small gonad's quality, (E) medium gonad's quality, (F) large gonad's quality.

and placed into 15 mL falcon tubes. Thereafter, blood samples were placed immediately in a small ice box before being transferred to the laboratory. Blood samples were centrifuged at 3000 × *g* for 5 min. Serum samples were then stored at - 20 °C until analysis. The steroids testosterone (T), progesterone (P) and 17β-estradiol (E_2) levels were measured by radioimmunoassay (RIA) according to the method of Fitzpatrick et al. (1986) using Immunotech kit (Hangzhou Estbiopharm Co. LTD, Hangzhoui, China). To cite the procedure in brief according to Feist et al. (2004), the plasma sample (100 µl) was extracted with diethyl ether (2 ml), then was vortexed and the aqueous phase was removed by freezing in liquid nitrogen. Extracts were re-suspended in 1 ml of phosphate-buffered saline with gelatin (PBSG). The antiserum was diluted in proportion of 1:30. A charcoal solution (6.25 g charcoal and 4.0 g dextran ether PBSG) was used for all assays in order to reduce nonspecific binding. In next steps, 1.0 ml of dextran-coated charcoal was added to each tube for the T and P assays and 0.5 ml for the E2 assay. The intra-assay coefficient of variation for all assays was less than 5%. The final reactions placed in each hormone-exclusive streptavidin (1.0 µg/ml) coated plates. Finally the levels of calibrators and real samples have been measured in 450 nm with gamma counter (LKB, Finland).

Measurement of serum biochemical parameters

Total protein analyzed by Biuret colorimetric method (Bionic, Italy), cholesterol determined by enzymatic colorimetric method (Man Company, Iran) and triglyceride has been measured by GPO-PAP Method (ZiestChem Diagnostics, Iran). Plasma glucose levels were determined by enzymatic colorimetric method using the commercial kit (Greiner, Bahlingen, Germany) and expressed as mg/dL.

Statistical analysis

Analysis of data was conducted using the SPSS 16.0 software. Before analysis, the normality of data was verified using the Shapiro-Wilk normality test. Statistical methods, including one-way ANOVA and Duncan's multiple range tests as well as Pearson's correlation were used at the level of 0.05. Data were presented as mean ± SD.

Table 1 Morphometric data and percentage of examined 3-year old female Siberian sturgeon classified according to gonadal fat content and gonad's quality

	Gonadal fat content			Gonad's quality (gonad's size)		
	Small	**Medium**	**large**	**Small**	**Medium**	**large**
Weight (kg)	4.70±1.51	6.04±1.82	5.06±1.53	3.38±1.05 [b*]	**5.20±1.54 [a]**	**5.33±1.52 [a]**
Length (cm)	93.00±9.05 [ab]	100.40±7.92 [a]	86.33±12.50 [b]	84.20±9.44 [b]	**95.20±10.08 [a]**	**95.60±7.94 [a]**
Number of fish (%)	22 (73.3%)	5 (16.6%)	3 (10%)	5 (16.6%)	10 (33.3%)	15 (50%)

* Different letters in each groups of gonad quality and fat content (separately) show the significant difference (P <0.05).

Results

In the current study, the gonad's quality and gonadal fat of 30 Siberian sturgeons in the weight range from 2.5 to 8.6 kg were determined using ultrasonography. Fish were divided into small, medium and large fat content (Figure. 1) that based on the amount of fat associated with gonad, only 10% of the examined fishes were in large fat content state while 73.3% of fishes considered small fat content (Table 1). In this classification, 50% of all fishes were considered as large gonad quality, 33.3% as medium quality (Table 1). According to Table 1, the large and medium gonad's quality specimens had significantly larger sizes (weight and length) than small quality group in both weight and length ($P < 0.05$). At this age, 16.6% of fishes showed small gonad quality.

Pearson's correlation to determine the intercorrelation between biometrical parameters and hormonal values are shown in Table 2. Results showed that there was a significant positive relationship between length and triglyceride ($R = 0.571$, $P < 00.1$) and between weight and triglyceride ($R = 0.665$, $P < 0.000$) while showed a negative correlation with testosterone ($R = -0.363$, $P < 0.05$). No correlation between body weight and total protein, cholesterol and glucose was observed ($P > 0.05$). Furthermore, no correlation between body weight and E_2 was found ($P > 0.05$).

In all individuals, serum E_2 ranged from 0.43 to 0.94 ng/dL, testosterone from 21.50 to 65.50 ng/dL, and progesterone from 2.85 to 8.26 ng/mL. Gonad's size showed significant difference with serum testosterone (Figure 2A) ($P < 0.05$). It was higher (49.72 ng/dL) in small size gonad while the medium size once had the lowest value (36.26 ng/dL) ($P < 0.05$). Progesterone also showed the similar trend so that was the highest in the small gonad's size fish ($P < 0.05$). E_2 exhibited no significant differences among gonad's size different classifications ($P > 0.05$).

Serum sex steroid levels of 3-year Siberian sturgeon with different gonadal fat content were measured and illustrated in Figure. 2B. All steroid hormones showed no significant differences in fish with small, medium or large gonadal fat content ($P > 0.05$). In the small gonadal fat content, progesterone and testosterone were slightly higher than those of medium and large fat content, however the difference was not significant ($P > 0.05$).

In all studied fish, serum total protein ranged from 2.98 to 5.88 g/dL, triglyceride from 102.00 to 265.00 mg/dL, cholesterol from 45.00 to 89.00 mg/dL, and glucose from 31.00 to 52.00 mg/dL. On the other hand, large gonad's size fishes were characterized by higher triglyceride (172.26 mg/dL) with the coincidental lower total protein (3.92 g/dL) (Figure. 3A). In medium and large gonad's size groups, there was no significant difference in serum cholesterol (63.2-63.8 mg/dL) ($P > 0.05$) however; both groups had lower cholesterol than small group (73.8 mg/dL) ($P > 0.05$). From the results, all biochemical parameters did not show significant differences among small, medium and large gonad's size groups. Total protein, triglyceride, cholesterol and glucose content in serum of small, medium and large gonadal fat content were determined (Figure. 3A). All these parameters did not show any differences among the three groups ($P > 0.05$). Small gonadal fat content group had the non-significant lowest triglyceride levels (155.68 mg/dL) compared with large and medium fat content fishes ($P > 0.05$).

Discussion

At sturgeon farms, different rates of echo from gonad's tissue to transducer showed as bright spots on monitor indicating no uniformity in gonad's quality. In hypo echoic (darker) samples, the female gonad may be

Table 2 Pearson's inter-correlation among morphometric and biochemical parameters and sex steroid hormones in 3-year old female Siberian sturgeon

		Length	Weight	Progesterone	Testosterone	E2	Total protein	Cholesterol	Triglyceride	Glucose
Length	Pearson Correlation	1	.919**	-.131	-.195	-.065	.058	-.006	**.571****	.232
	Sig. (2-tailed)		.000	.489	.301	.733	.761	.976	.001	.217
	N	30	30	30	30	30	30	30	30	30
Weight	Pearson Correlation	.919**	1	-.285	**-.363***	.009	-.022	-.044	**.665****	.088
	Sig. (2-tailed)	.000		.127	.049	.964	.906	.816	.000	.644
	N	30	30	30	30	30	30	30	30	30
Progesterone	Pearson Correlation	-.131	-.285	1	.859**	-.543**	.047	.160	-.207	.226
	Sig. (2-tailed)	.489	.127		.000	.002	.806	.399	.272	.230
	N	30	30	30	30	30	30	30	30	30
Testosterone	Pearson Correlation	-.195	-.363*	.859**	1	-.725**	.221	.232	-.196	.297
	Sig. (2-tailed)	.301	.049	.000		.000	.241	.218	.300	.112
	N	30	30	30	30	30	30	30	30	30
E2	Pearson Correlation	-.065	.009	-.543**	-.725**	1	-.182	-.281	-.157	-.304
	Sig. (2-tailed)	.733	.964	.002	.000		.335	.132	.406	.102
	N	30	30	30	30	30	30	30	30	30
Total protein	Pearson Correlation	.058	-.022	.047	.221	-.182	1	-.005	.369*	.252
	Sig. (2-tailed)	.761	.906	.806	.241	.335		.980	.045	.179
	N	30	30	30	30	30	30	30	30	30
Cholesterol	Pearson Correlation	-.006	-.044	.160	.232	-.281	-.005	1	.296	.506**
	Sig. (2-tailed)	.976	.816	.399	.218	.132	.980		.112	.004
	N	30	30	30	30	30	30	30	30	30
Triglyceride	Pearson Correlation	.571**	.665**	-.207	-.196	-.157	.369*	.296	1	.423*
	Sig. (2-tailed)	.001	.000	.272	.300	.406	.045	.112		.020
	N	30	30	30	30	30	30	30	30	30
Glucose	Pearson Correlation	.232	.088	.226	.297	-.304	.252	.506**	.423*	1
	Sig. (2-tailed)	.217	.644	.230	.112	.102	.179	.004	.020	
	N	30	30	30	30	30	30	30	30	30

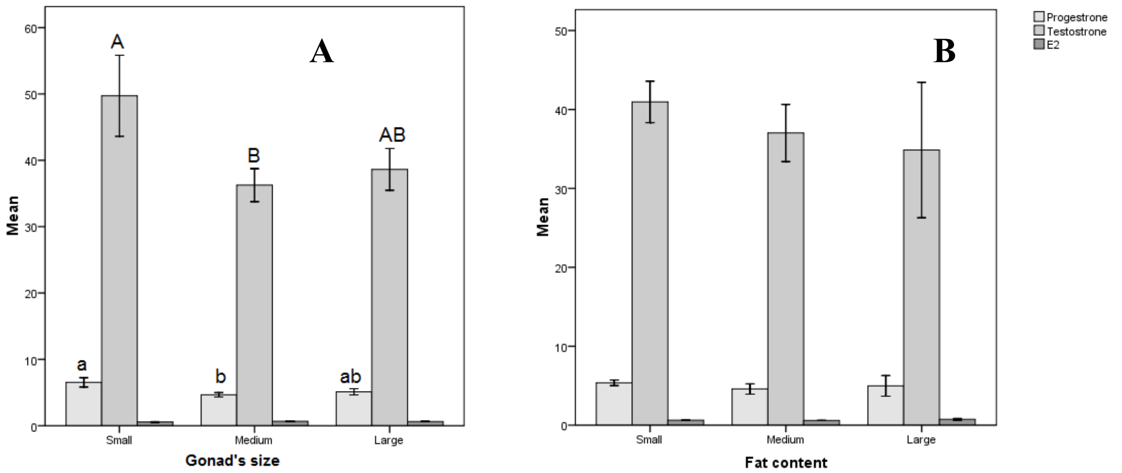

Fig. 2 Serum testosterone and E_2 (ng/dL) and progesterone (ng/mL) levels in 3-year old Siberian sturgeon with different gonad's quality (A) (small, medium, and large) and gonadal fat (B) (small, medium, and large). All data were expresses as mean ± SD (n = 30).

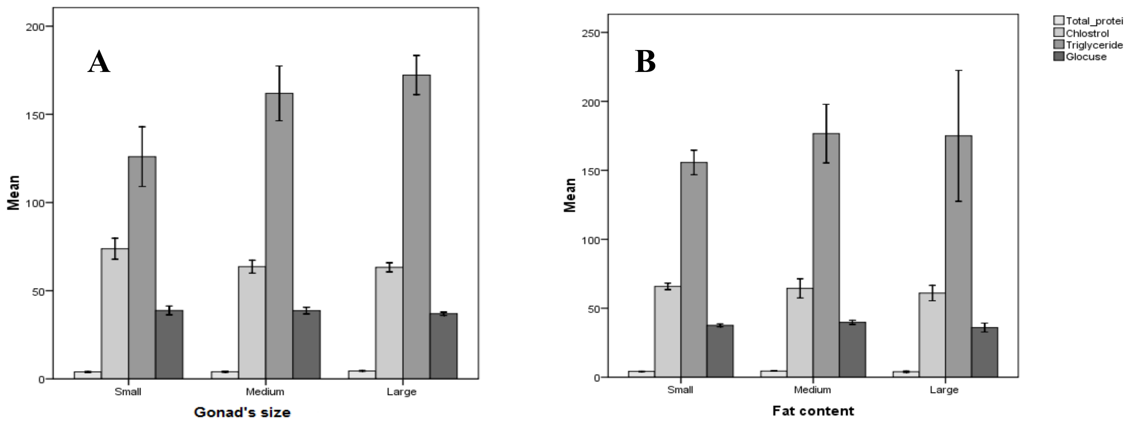

Fig. 3 Serum total protein (g/dL), triglyceride, cholesterol and glucose (mg/dL) levels in 3-year old Siberian sturgeon with different gonad's quality (A) (small, medium, and large) and gonadal fat (B) (small, medium, and large). All data were expresses as mean ± SD (n = 30).

wrongly regarded as the male gonad. Therefore, this is one reason of not expecting to have 100% accuracy of sturgeon sex using ultrasonography, and consequently the sonogram's quality and sonographer's expertise both directly influences on gonadal qualifications. In the current study, larger fishes showed medium and large gonad's quality determined by ultrasonography images (Table 1), however, the differences in size between the medium and large gonad's quality groups were not significant. Additionally, at this age, smaller fish had small gonad's quality (Table 1). Similarly, the positive relationship between the size of the fish and the size and strength of the sexual organs have been proven in many studies (Gholampour et al. 2011; James and Sampath 2004). Thus ultrasound method confirms the bigger fishes in the same age have better gonad's quality in Siberian sturgeon. Colombo et al. (2007) and Tripp (2007) reported a significant positive relationship between fecundity and weight of the shovelnose sturgeon (*Scaphirhynchus platorynchus*) harvested from middle of Mississippi River. Also, some positive relationships were observed in Siberian sturgeon (*A. baeri*) breeders for selection of suitable fish (Williot et al. 1991).

The difference between steroid hormones and biochemical parameters altogether with ultrasound observations in sturgeon fishes are an interesting area to be investigated accordingly. The difference between ultrasound and steroid hormones in sex determination has been investigated in some studies (Craig et al. 2009, kazemi et al. 2014, Du et al. 2017). In our study, gonad's quality showed significant difference with serum testosterone and progesterone among groups, being higher in small gonad's quality group. In contrasts, serum T, P and E_2 levels were not differentiated with gonadal fat content in 3-year old Siberian sturgeon. In small gonadal fat content group, testosterone was slightly higher, however, this difference was not significant. Results from the Falahatkar (2013) showed no differences in serum T and

P at the migratory stages in fish and E_2 levels were correlated with oogenesis and decreased significantly from the perinucleolus to the migratory stage. Some findings showed that T levels increased more rapidly before final maturation (Barannikova et al. 1999). Therefore, as the fish were in the early stages of sexual maturation as Seberian sturgeon in the present study, steroid hormone levels have not fluctuated very much. The results also showed that as the size of the body increases, the amount of T decreases but length and weight were not correlated by the E_2 and P. However, Hedayati and Bagheri (2010) showed that there was a significant negative correlation between length and weight with progesterone in 4-year cultured beluga (*H. huso)* in brackish water. In other specis, the study of Imanpour et al. (2018) on *Esox lucius* showed that the levels of E2 and T increased with increasing fish length.

The results showed that body length and weight were positively correlated with triglycerides, while it was not correlated with other biochemical biomarkers (total protein, triglyceride, cholesterol and glucose). This is while Shamoshki (2014) stated that the values of biochemical parameters in different sizes of fish differ significantly. Various studies have shown that biochemical parameters levels increase with increasing age (Sano 1960; cited in: Rehulka 2000), however, in fish belonging to the same species, differences in biochemical parameters can be seen, due to differences in food type (Rehulka 2000). It must be also considered that a progressive trend in triglyceride value from small to large groups (Fig. 3 A) could be seen in gonad's quality that was not significantly different but could be potentially followed in further studies to prove that the bigger fishes in the same age, has better gonad's quality and higher values of triglyceride associated in blood, as a solely promising biochemical biomarker found in the present study. However, In order to make more comprehensive results, getting the samples from different farms and intervals will be definitely recommended for next studies.

Conclusions

In conclusion, it can be pointed out that the most of ultrasonography studies focused on using sex determination of sturgeon, but our study in gonad's quality and gonadal fat content associated with sonogram images is considered as an initiation in practical perspective of this area that could provide useful information for sturgeon farms to focus on smaller sizes of specimens in the same age with weaker gonad's quality expected. Then sorting the bigger individuals in weight and length to have better stocks for both reproduction and caviar production purposes could be better managed. Thus, selecting larger females in the same age to become more productive will be more beneficial for sturgeon farms.

As triglyceride exhibited a positive significant relationship with fish size, and a positive trend has also been detected in large gonad's quality group rather than small group, when there is no ultrasound available in a farm, the triglyceride determination in fish serum might be a substitute good tool to differentiate the better gonadal quality in female, however needed further studies in future.

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Conflict of interest The authors declare that they have no conflict of interest.

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