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DEVELOPMENT OF MALE RUSSIAN STURGEON (*ACIPENSER GUELDENSTAEDTII*) AND HYBRID (*ACIPENSER BAERII X ACIPENSER GUELDENSTAEDTII*) GONADS REARED IN NET CAGES

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Abstract

The study was performed with male Russian sturgeon (*Acipenser gueldenstaedtii*) individuals aged five and seven years and hybrid of Siberian and Russian sturgeon (F_1 *Acipenser baerii x Acipenser gueldenstaedtii*) aged 7 years, reared in a cage farm, located in a warm water reservoir in Bulgaria. Ultrasound monitoring was used to study the development of the testes in the spring - winter period. What was found is that, unlike the Russian sturgeon, in the hybrid, during the vegetation period, the testes changed significantly, increasing all the studied indicators (height, girth and area). In the spring, the highest testicular height was found in seven-year-old Russian sturgeon, and the difference (25%) with the hybrid of the same age was significant (P <0.05). There are significant differences in favor of Russian sturgeon in terms of girth (22%; P <0.001) and testicular area (54%; P <0.001). At the end of the vegetation period, a significant difference (16%; P <0.05) in testicular height was found between Russian sturgeon of different ages, in favor of older fish. There is no significant difference between the different age categories in terms of the girth and area of the testes. Although the differences in the size of the gonads between the Russian sturgeon and the hybrid are not significant, there is a tendency for compensatory development of the gonads in the latter. In spring, the gonads of the hybrid were less developed than those of the Russian sturgeon of both ages, and in winter they were better developed. Our study shows that in an industrial cage farm environment, the male hybrids of Siberian and Russian sturgeon (F1) are not inferior in their sexual development to the Russian sturgeon.

Keywords: sturgeons, aquaculture, gonad development, sexual development.

INTRODUCTION

In the past, natural sturgeon populations played an important economic role as a food resource for the people inhabiting the Danube Basin region (Hochleithner and Gessner, 2012). However, natural populations of all sturgeon species are severely affected by overexploitation (Ivanova et al. 2017; Maltsev, 2009, etc.). Due to anthropogenic impact, species such as Atlantic sturgeon (*A. sturio*) and Ship sturgeon (*A. nudiventris*) are now considered extinct in the Danube region, while the populations of Beluga (*H. huso*), Russian sturgeon (*A. gueldenstaedtii*), Stellate sturgeon (*A. stellatus*) and Sterlet (*A. ruthenus*) were significantly reduced (Reinartz 2002; Williot et al., 2002; Lenhardt et al., 2006).

Russian sturgeon (*A. gueldenstaedtii*) inhabits the Black, Azov and Caspian Sea, as well as their adjoining rivers, and in recent years has been introduced en masse into aquaculture, following a drastic decline in natural populations and an increase in the caviar price of the same species (Hurvitz et al. ., 2008). Along with Russian sturgeon, hybrids (for example Russian and Siberian sturgeon one) are also valuable for sturgeon aquaculture (Havelka and Arai, 2018). Russian and Siberian sturgeon hybrid is characterized by a high growth rate, which significantly exceeds that of the Russian sturgeon at all stages of its development (Efimov, 2004). The cultivation of various Siberian sturgeon hybrids is of practical importance for industrial breeding (Chebanov et al., 2018).

The development of sturgeon farming is important for the conservation of endangered natural populations (Burtsev, 2013). It is necessary to know the development patterns of the cultivated species for efficient reproduction in a full-system aquaculture farm. In this regard we set a goal to study the development of Russian sturgeon and hybrid (Siberian x Russian sturgeon) testes raised in a super-intensive cage farm by using ultrasound methods.

MATERIALS AND METHODS

The study was conducted with Russian sturgeon (*A. gueldenstaedtii*) male individuals at five (Ag5) and seven years of age (Ag7); Siberian and Russian sturgeon hybrid (F_1 *A. baerii x A. gueldenstaedtii*) at the age of seven (Hy7) in spring. The monitoring was carried out in the same groups during the winter: Russian sturgeon aged six (Ag5 +) and eight summers old (Ag7 +); and hybrid of Siberian and Russian sturgeon at the age of eight summers (Hy7 +). The first scan was performed in May 2017, and the second in December 2017. In both periods, 10 individuals from each group were studied (n = 60).

The fish have been grown on a farm for super intensive production in cages localized in the reservoir of Kardzhali since they were one summer old. By type, the reservoir refers to large, deep ones - its area is 16.07 km^2, volume 532.9 $x10^6 m^3$. The dam of Kardzhali is located in Southeastern Bulgaria located at 41° 37 'N latitude and 25° 20' E longitude, falling in the South-Bulgarian climate zone, East Rhodopian climatic region. The average altitude is about 280 m.

Fish of different species and age groups were reared in separate cages with dimensions 8x8 m with a water depth of 6 m from the surface. The net of the cages is double, made of polyamide. Feeding is performed manually with specialized granulated feed for sturgeon.

Testicular development was monitored by ultrasound examination of live fish that did not participate in the breeding campaign. Mindray DP 50 ultrasound scanner with a 75L38EA linear transducer (5-10 MHz) was used. The testicular height (cm) in frontal view was measured with special options of the ultrasound scanner; girth (cm) and area (cm^2) in a transverse view. The scan was performed on the line just above the abdominal bone scutes, in the area between the third and fourth, counted from the abdominal fin to the head. IBM SPSS Stasistics 21 was used for statistical data processing.

RESULTS AND DISCUSSION

A collection of 60 ultrasound images was collected during the study, from which we selected the most characteristic ones for each group (Fig. 1-6). Sturgeons are characterized by a lack of well-defined sexual dimorphism, which significantly complicates the work of artificial reproduction. When grown from the earliest stages of life in unusual conditions in super-intensive farms their sexual development raises many questions related to the morpho-functional formation and functioning of their reproductive system. The processes of gonadal development in Russian sturgeon have been poorly studied (Kamaszewski et al., 2016). According to McGuire et al. (2019) a better understanding of the chronology of farmed fish gonad development can help predict the sex ratio and maintain demographically stable populations. Omoto et al. (2002) report that there is no detailed information on Sturgeon gonads development.

Fig. 1 shows a frontal (1a) and transverse (1b) ultrasound image from a scan of a male Russian sturgeon gonads at the age of 7 years. In the frontal image, the height of the testis was measured (Dist 1.54 cm).

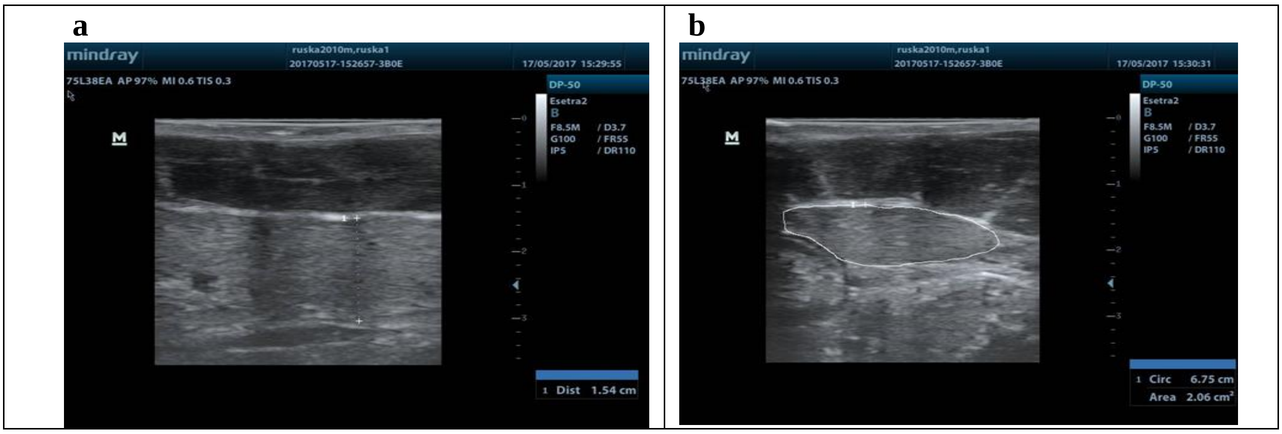

***Fig. 1**. Ultrasound images of Ag_7 (spring season), (a) frontal view (b) transverse view*.

The photo clearly shows the homogeneous structure of the generative part, as well as the strong hyperechogenicity. The hyperechogenic serous membrane stands out very clearly. At the lower end, a very small area with an aechogenic echostructure can be seen. This is testicular fat. The next image of the same individual shows the location of the testis on transverse view (1b), which has a homogeneous structure and hyperechoic character here too. The testicular girth (Circ 6.75 cm) and its area (Area 2.06 cm^2) were measured with the automatic option for various real-time measurements. In the same species at the age of 6 years (Fig. 2), in frontal view (2a) the muscles have various echogenicity with different nuances. The serous membrane is clearly visualized due to its hyperechogenic structure. The testis is very well defined with a pronounced homogeneous shape, with clearly defined smooth contours. The photo shows a very small part of the testis with an aechogenic character - testicular fat. The measured testicular height is 1.61 cm (Dist 1.61 cm), which is slightly more than the frontal image of the same group of individuals from the spring season. Transverse scanning of the same individual (2b) shows the same characteristics as the frontal image. The testis here is visualized with a clear homogeneous shape with smooth, well-defined contours too. The measured girth (Circ 7.88 cm) and area (Area 3.35 cm^2) of the testis are larger than the transverse image of the same group of individuals during the spring season.

A frontal ultrasound image (3a) from a scan of the gonads of a 5-year-old Russian sturgeon is shown in Fig.3. The height of the testis was measured (Dist 0.95 cm). The generative part has a pronounced homogeneous structure and hyperechogenicity. At the lower end there is a long strip with aechogenic echostructure, which is testicular fat. The musculature covers a large part of the photo and is of mixed echogenicity. In the transverse scan of the same individual (3b), the testis is visualized with a clear homogeneous shape with smooth, well-defined contours. The girth (Circ 6.11 cm) and area (Area 1.79 cm²) of the testis are smaller than those of male Russian sturgeon individuals aged 7 years old. The musculature covers a large part of the photo and is of mixed echogenicity. The muscle fibers are visualized with medium brightness, and their alternating walls, separated by connective tissue, appear on the screen as narrow sloping, almost vertical stripes and have a brighter hue than the muscles. After the examinations carried out during the spring season, such were performed on the same group of individuals during the autumn-winter season as well (Fig. 4). The height of the testis is 1.28 cm (Dist 1.28 cm), and here its homogeneous echostructure is no exception. The girth (Circ 6.35 cm) and the area (Area 2.10 cm^2) are plotted, as in all individuals so far.

Ultrasound examinations were performed on hybrids along with the Russian sturgeon. A demonstration of the results of the spring study in hybrids (Ab X Ag) at 7 years of age is shown in fig. 5. The testis has a clearly distinguishable homogeneous structure. Its height is 1.61 cm (Dist 1.61 cm) The musculature has a mixed echogenic character, with almost no vertical stripes, which represents the walls of the muscle fibers, separated by connective tissue.

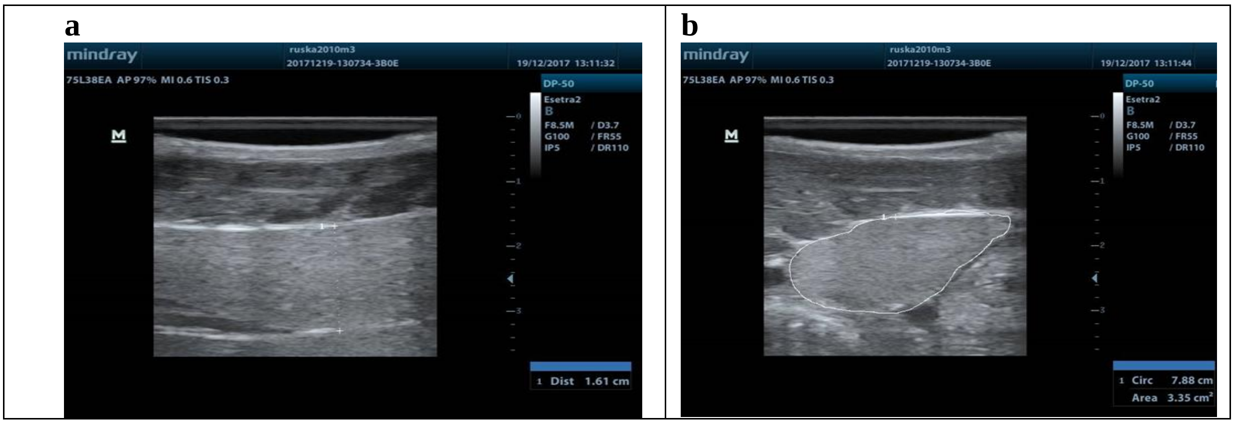

Fig. 2. *Ultrasound images of Ag_7 (winter season), (a) frontal view (b) transverse view*.

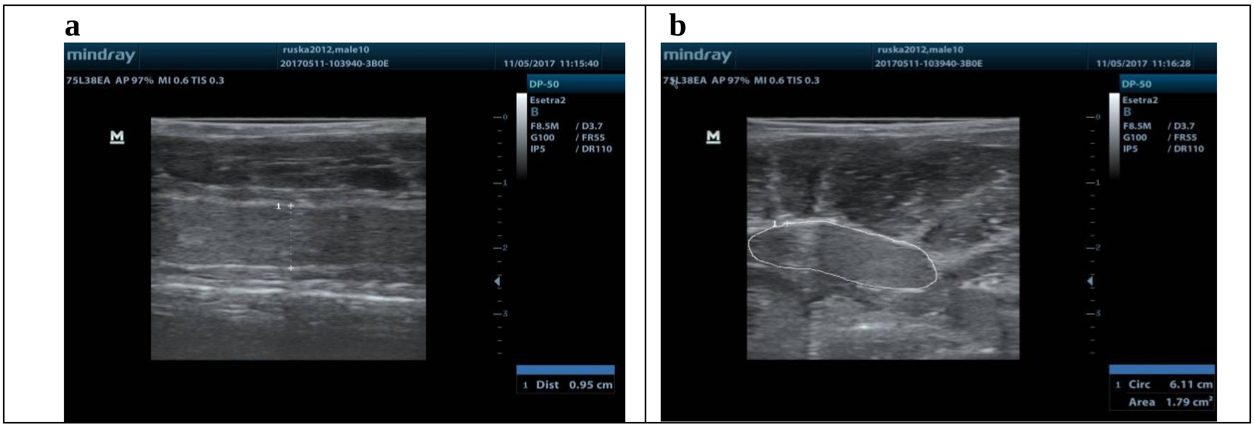

Fig. 3. *Ultrasound images of* Ag_5 *(spring season), (a) frontal view (b) transverse view.*

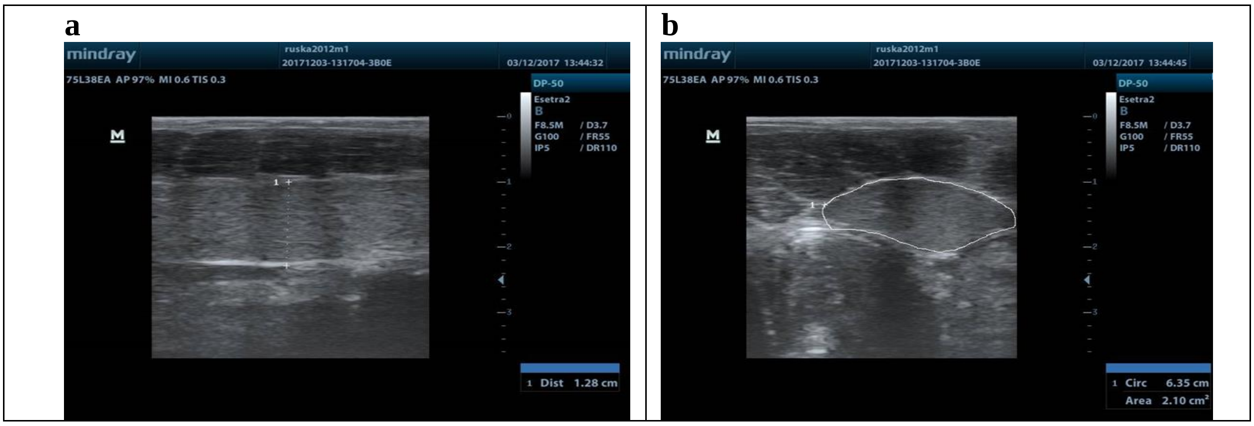

Fig. 4. *Ultrasound images of* Ag_5 *(winter season), (a) frontal view (b) transverse view.*

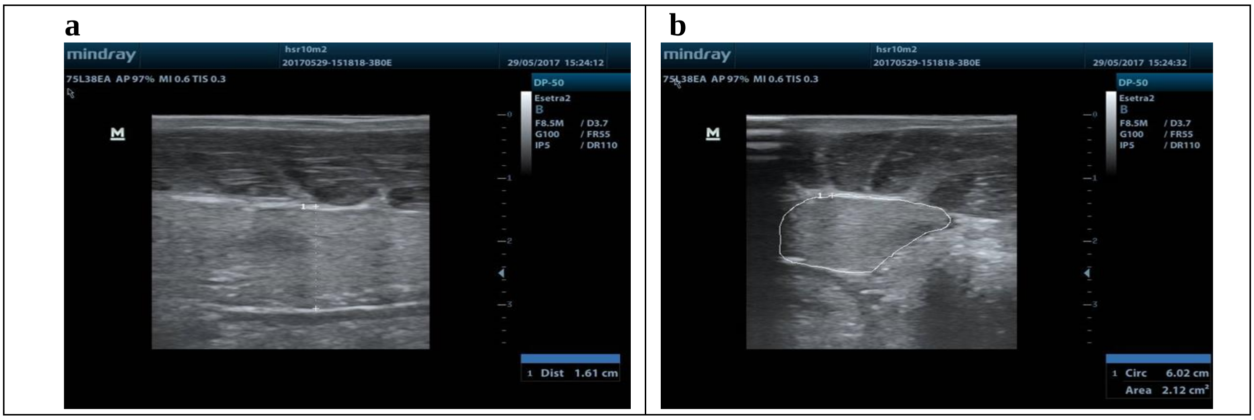

Fig. 5. *Ultrasound images of* Hy_7 *(spring season), (a) frontal view (b) transverse view.*

In the transverse view of the individual (5b) the parameters from the frontal one are confirmed. The girth of the testis is 6.02 cm and its area is 2.12 cm^2. In the autumn-winter ultrasound examination of male hybrids from the same group, the same signs were observed, but there was a difference in the height of the testis, as shown in Figure 6, where a frontal ultrasound image (6a) of a hybrid (Ab X Ag) is shown. The homogeneous structure of the testis stands out quite well. Its height is 1.94 cm (Dist 1.94 cm). The transverse image (6b) shows that the girth (Circ 8.62 cm) and the area (Area 4.64 cm²) are larger than in the spring.

In the Russian sturgeon of both ages, no significant differences were found in the size of the generative part of the testis at the beginning and end of the vegetation period (Table 1).

In contrast to the Russian sturgeon, in male hybrids during the growing season all the studied indicators increased (Figure 1). Testicular height increased by 40.5% ($P < 0.05$); the girth by 28.1% ($P < 0.01$); and the area practically twice ($P < 0.01$).

In the spring, the highest testis was the one in seven-year-old Russian sturgeon, and the difference (25%) with hybrids of the same age was significant ($P < 0.05$).

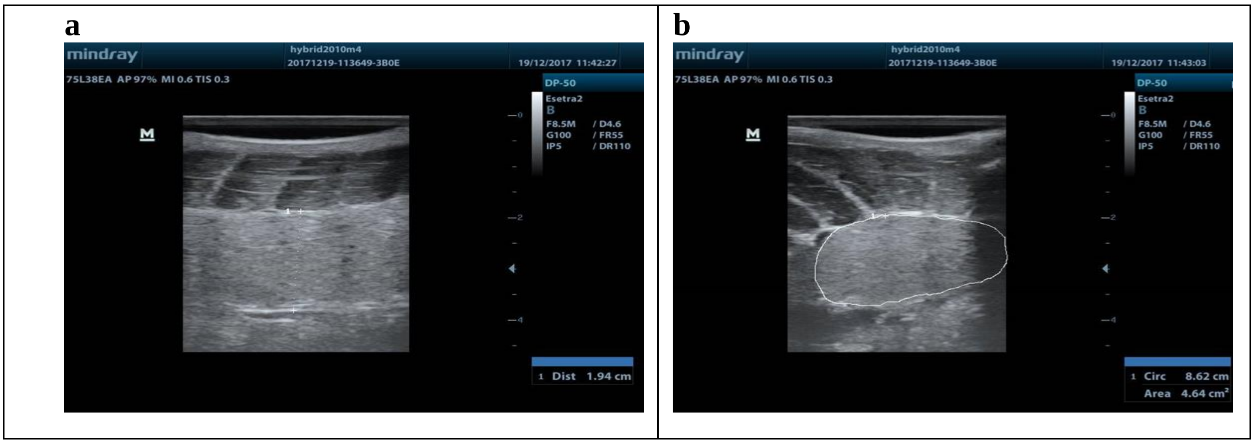

Fig. 6. *Ultrasound images of Hy_7 (winter season), (a) frontal view (b) transverse view.*

Table 1. *Testicular characteristics of Russian sturgeon and Siberian and Russian sturgeon hybrid, of different ages during different season.*

Indices	Genotype / Age					
	Ag_5	Ag_{5+}	Ag_7	Ag_{7+}	Hy_7	Hy_{7+}
	Season					
	Spring	Winter	Spring	Winter	Spring	Winter
Height, cm						
ΔX	1.31	1.31e	1.45c	1.52e	1.16ac	1.63a
±Sx	0.07	0.04	0.11	0.10	0.12	0.15
CV	15.79	9.96	22.38	19.36	30.45	27.34

Girth, cm						
ΔX	6.86	6.96	6.81d	6.96	5.69bd	7.29b
±Sx	0.33	0.30	0.21	0.28	0.21	0.47
CV	14.30	12.74	9.08	12.27	11.04	19.29
Area, cm^2						
ΔX	2.52	2.68	2.51d	2.64	1.63bd	3.17b
±Sx	0.23	0.27	0.17	0.25	0.14	0.42
CV	26.97	30.05	20.80	28.01	25.89	40.16

Differences between the values denoted by the same symbols in the rows are significant: d– p < 0.001; b– p < 0.01; a;c;e – p < 0.05

The same applies to the girth with the corresponding difference of 22% (P <0.001). There was no significant difference in the area of the testis in the Russian sturgeon of the two age categories. In seven-year-old individuals, the area was 54% larger than in hybrids of the same age (P <0.001).

At the end of the vegetation period, a significant difference (16%; P <0.05) was found in the height of the testes between Russian sturgeons of different ages, in favor of seven-year-old fish. There is no significant difference between the different groups in the girth and area of the testes. Although there are no significant differences in gonad size between the Russian sturgeon and the hybrid, it is noteworthy that in the spring season the latter gives way to the Russian sturgeon, and in the end, the gonads of the hybrid outpace those of the Russian sturgeon.

A number of authors study the qualities of different hybrids in sturgeon breeding (Ponomareva et al., 2019). Hybridization increases heterozygosity, reduces the effects of recessive lethal genes (Whitlock et al., 2000). The study by Shivaramu et al., (2019) showed that in artificial breeding, hybrids of Siberian and Russian sturgeon had higher survival and growth than purebred fish. Safronov et al. (2016) studied several hybrids in terms of growth rates, morphological features, and gonadogenesis, comparing them with their parental forms. The authors point out that a number of hybrids have better productivity indicators and their use allows to improve the fish-biological indicators in sturgeon farming.

Our study shows that in the conditions of an industrial cage farm located in a large warm water reservoir in Bulgaria, the male individuals of the Siberian and the Russian sturgeon hybrid are not inferior in their sexual development to one of the parental forms, the Russian sturgeon.

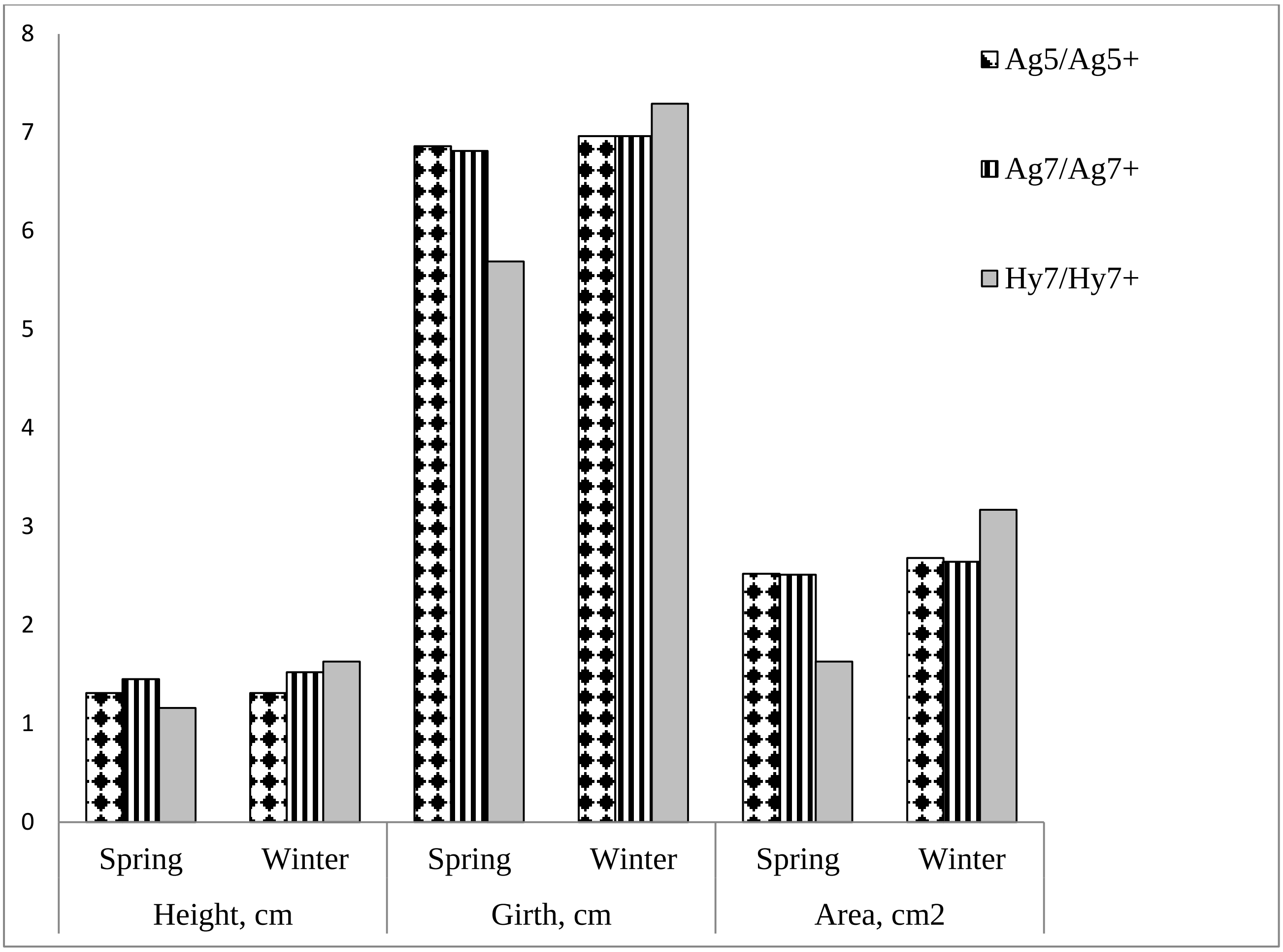

Fig. 7. *Testicular development dynamics in Russian sturgeon (A. gueldenstaedtii) and a hybrid of Siberian and Russian sturgeon (F1 A. baerii x A. gueldenstaedtii) during different seasons*.

CONCLUSIONS

As a non-invasive method, ultrasound provides a good opportunity for monitoring testicular development in sturgeon fish. When the image is formed during the ultrasound scan, the testicular tissue can be easily differentiated. The part of the testes is hyperechoic. The fatty part is insufficiently or poorly developed on the medial side and is practically difficult to see. The borders of the testes are smooth, and their bright hyperechoic tunic is clearly visible. In contrast to Russian sturgeon, in the hybrid during the vegetation period the testes change significantly, increasing all the studied indicators (height, girth and area). In the spring, the highest testicular height was found in seven-year-old Russian sturgeon, and the difference (25%) with the hybrid of the same age was significant (P <0.05). The same applies to the girth (22%; P <0.001) and the area (54%; P <0.001). At the end of the vegetation period, a significant difference (16%; P <0.05) between Russian sturgeon individuals of different ages was found in testicular height, in favor of older fish. There is no significant difference between different age categories in terms of the girth and area of the testes. Although the differences in size of the gonads between Russian sturgeon and the hybrid are not significant, there is a tendency for compensatory development of the gonads in the latter. In the spring, the hybrid gives way to the Russian sturgeon, and in the end the gonads of the hybrid get ahead of those of the Russian sturgeon. Our study shows that in an industrial cage farm environment, the male individuals of the hybrid of Siberian and Russian sturgeon (F1) are not inferior in their development to one of the parental forms, the Russian sturgeon.

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