Bulgarian Journal of Agricultural Science, 28 (Suppl. 1) 2022
Agricultural Academy

# Characteristics of a hybrid (F1 *A. baerii* x *A. gueldenstaedtii*) sperm production at different ages when grown on an industrial cage farm

**Stanimir Bonev[1*], Boyko Georgiev[2], Lyudmila Nikolova[1], Paulina Taushanova[2]**

[1]*Agricultural University, Faculty of Agronomy, Department "Animal science", BG-4000 Plovdiv, Bulgaria;*
[2]*Institute of Biology and Immunology of reproduction "Acad. K. Bratanov", BAS, Sofia, Bulgaria.*
[*]*Corresponding author:* dr_bonev@yahoo.com

## Abstract

Bonev, S, Georgiev, B, Nikolova, L., Taushanova, P. (2022) Characteristics of a hybrid (F1 *A. baerii* x *A. gueldenstaedtii*) sperm production at different ages when grown on an industrial cage farm. *Bulg. J. Agric. Sci.*, *28 (Supplement 1)*, 164–171

The study was performed with male hybrid individuals (F1 *Acipenser baerii* x *Acipenser gueldenstaedtii*) aged seven, eight and nine years, grown on a cage farm located in a warm water dam. No significant differences were found between age groups in ejaculate volume and sperm concentration. Motility analysis showed that sperm in the oldest individuals had the highest values ($p < 0.05$) of VCL, VSL, VAP, ALH and BCF, while the differences in indicators were not significant between the hybrids at 7- and 8 years of age. The relative proportion of rapid sperm in the hybrid semen of varies from 0 to 96.90%; of medium sperm from 1.0 to 46.74%; slow from 0.28 to 75.30%; static from 0 to 84%. The relative share of spermatozoa with non progressive motility varies from 15.30 to 89.70%, and those with progressive motility – from 0.10 to 29.85%. The oldest fish had significantly ($p < 0.05$) the best characteristics of sperm motility – the highest share of rapid and those with progressive motility; lowest of the slow and static. Age affects the enzymatic activity of sperm. In 7- and 9-year-old fish, a significant difference was found in AP, GGT, CK in water extract, and in LDH in triton extract. The water extract in 9-year-olds has higher levels of AP (7 times; $p < 0.05$) and GGT (3.2 times; $p < 0.05$), while in 7-year-olds fish the values of CK are higher (2.3 times; $p < 0.05$). In LDH, the difference (1.5 times) is also in favor of 7-year-old individuals, but it is not significant. In the triton extract only AP levels are higher (over 30%) in 9-year-old fish, but the difference is not significant. For the other indicators (GGT, CK and LDH) the difference is in favor of the younger fish, respectively 4.6 %, 22.1 % and 2.9 times, and for the latter it is statistically significant ($p < 0.05$).

*Keywords:* sturgeon semen, enzyme activity, spermatozoa velocity.
*Abbreviations: CASA – Computer-assisted sperm analysis; VCL – curvilinear velocity; VSL – straight line velocity; VAP – average path velocity; LIN – linearity; STR – straightness; WOB – wobble; ALH – amplitude of lateral head displacement; BCF – beat/cross frequency; AP – Alkaline Phosphatase; GGT – Gamma-glutamyl Transferase; CK – Creatine Kinase; LDH – Lactate Dehydrogenase.*

## Introduction

The state of sturgeon populations in the world is of particular concern. The anthropogenic factor causes huge damage to the natural populations of sturgeon species. A number of measures have been taken to protect and restore them (Bloesch et al., 2006). The global trend for the conservation of natural sturgeon populations is their introduction into new areas for industrial breeding in artificial conditions (Kuderskiy, 2015). Given the absolute ban on sturgeon fishing from natural populations (MZH, 2016), meeting the needs of valuable sturgeon products will be provided only by sturgeon aquaculture. At the same time, super-intensive sturgeon farming is a relatively new activity and has many unresolved issues, especially in the field of breeding and reproduction. In industrial fish farming, the efficiency of artificial reproduction is directly related to the assessment of the quality of sperm production (Alavi et al., 2012; Hajirezaee et al., 2010).

The quality of fish sperm is important for optimizing production, as one of the determining signs of sperm function is its enzymatic activity (Kowalski & Cejko, 2019). Enzymes in fish semen have been less studied compared to mammals (Aramli et al., 2013).

Given the lack of information on sperm quality and enzyme activity in sturgeon farmed in industrial aquaculture farms, we aimed to study the main quantitative and qualitative characteristics of Siberian and Russian sturgeon hybrid sperm raised in a super-intensive cage farm.

## Materials and Methods

The study was performed with a hybrid (F1 *Acipenser baerii x Acipenser gueldenstaedtii*) at seven, eight and nine years of age. The fish were randomly selected from industrial herds of producers, raised in a super-intensive cage farm, situated in a large warm water dam, located in Southeastern Bulgaria at 41° 37 'N latitude and 25° 20' E longitude. The region falls within the southern Bulgarian climatic zone, Eastern Rhodope climatic region. The average altitude is about 280 m. Fish were kept under identical conditions throughout the study period. A specialized granular fish feed was used for feeding.

The study was conducted over three consecutive years. Upon receipt of the semen, the volume of ejaculate was immediately measured, after which the semen was transported in refrigerated bags in a laboratory for biochemical analysis. Sperm concentration was determined by the CASA system; total motility; VCL – curvilinear velocity; VSL – straight line velocity; VAP – average path velocity; LIN – linearity; STR – straitness; WOB – Wobble; ALH – amplitude of lateral head displacement; BCF – beat/cross frequency; the percentage of rapid, medium, slow and static sperm; sperm with non-progressive and progressive motility.

The activity of LDH (lactate dehydrogenase), AP (alkaline phosphatase), GGT (gamma glutamyltransferase) and CK (creatine kinase) was analyzed. To remove the seminal plasma, ejaculate was centrifuged at 3000 rpm (Hermile Labor Technik, Z326 K) for 10 min. Enzyme activity was performed by biochemical analyzer Mindray BA88A, using the following reagents:

GGT-Tris buffer 100 mM pH 8.25, glycil-glycine 100 mM, L-Glutamyl-4-nitroanilide 4mM, wavelength 405 nm;

AP-DEA buffer pH 9.8 1M, $MgCl_2$ 0.5 mM, 4-Nitrophenilphosphate 10 mM, wavelength 405 nm;

CK-Imidazole buffer 29 mM pH 6.50, creatine phosphate 30 mM, glucose 20 mM, N-Acetyl-L-cysteine 20 mM, magnesium acetate 10 mM, EDTA 2 mM, ADP 2mM, NADP 2mM, AMP 5mM, Di(adenosine-5) pentaphosphate 12 MikroM, glucose-6-phosphate-dehydrogenase > 3kU/l, hexokinase > 3kU/l, wavelength 340 nm;

LDH-phosphate buffer pH7.50 50 mM, sodium pyruvate 0.60 mM, NADH 0.18 mM, 340 nm wave length.

Sperm pellet, received after seminal plasma removal was resuspended with 0.9% saline and centrifuged at 3000 g for 10 min at 4° C. The procedure was repeated 3 times. After last centrifugation, the pellet was resuspended with distilled water and freeze at -20° C overnight. On the next day, the samples were sonicated by ultrasound 3 x 10 sec and centrifuged at 12000 g, 30 min. at 4° C. Received supernatant, containing water soluble proteins was removed and analyzed for enzyme activity. The pellet was resuspended with 1% triton X100 and centrifuged at the same condition. Supernatant was analyzed for membrane connected proteins.

IBM SPSS Stasistics 21 was used for statistical processing.

## Results and Discussion

Indicators of the sperm production quality in hybrids of different age are presented in Table. 1.

The volume of ejaculate in the studied hybrid varies from 36 to 150 ml; sperm concentration from 749.60 to 2787.36 x $10^6$ / ml; total motility – from 16 to 100%. No significant differences were found between age groups in ejaculate volume and sperm concentration. The highest average volume was obtained in 8-year-old fish (104 ml), with differences in the indicator with 7- and 9-year-old fish being 23.81 and 14.49%, respectively. The highest concentration is in 7-year-old fish, which have the smallest ejaculate volume. The difference in concentration between the youngest fish and those at 8- and 9 years of age is 26.56 and 23.21%, respectively, and between 8- and 9-year-old fish the difference is only 2.7%. The highest sperm motility was found in 9-year-old fish (over 99%), as the differences with other age groups were significant, respectively with 7-year-olds – 28.77% ($p < 0.05$), and with 8-year-olds it reached 53.44% ($p < 0.05$). In the oldest fish, the highest motility is combined with a relatively good ejaculate volume and sperm concentration. In the same group the lowest level of variation of motility was found, as the minimum motility is high enough – 97.8%.

According to Dzyuba et al. (2017) characteristics of semen largely depend on the species of fish, and in artificial conditions it is possible to obtain large amounts of semen (from several ml to several hundred ml) with a concentration of 0.02 to 10 x $10^9$ / ml and motility up to 100%. In sturgeon species, compared to the families Cyprinidae and Percidae, sperm have a larger head and tail, and motility is longer (Akimochkina, 2010). Concentration and motility vary in different studies in the same species and in different species. Thus, Horokhovatskyi et al. (2018) obtained 89.3% sperm motility for Sterlet, and Fedorov et al. (2017) also for Sterlet – 66%. In a study of sperm concentration and motility in trout, the following values were found: 2.6 x $10^9$ / ml. (2600 x $10^6$ / ml) at a motility of 81.16% (Sadeghi et al., 2013) and 2.4 x $10^9$ / ml at a motility of 80.63% (Sadeghi et al., 2020). Shaluei et al. (2017) conducted two experiments with Persian sturgeons, with the first concentration being 1,880 x $10^9$ / ml (1880 x $10^6$ / ml) and the second 2.09 x $10^9$ / ml (2090 x $10^6$ / ml).

**Table 1. Indicators characterizing the hybrid sperm quality**

| Parameters | Indicators | | |
|---|---|---|---|
| | Volume, ml | Motility, % | Concentration, $1.10^6$/ml |
| 7 years old (n = 5) | | | |
| X | 84.00 | 77.36 c | 1956.08 |
| ±SE | 15.26 | 8.37 | 223.09 |
| CV | 27.73 | 44.84 | 47.81 |
| min | 45.00 | 16.00 | 749.60 |
| max | 100.00 | 98.70 | 2787.36 |
| 8 years old (n = 3) | | | |
| X | 104.00 | 64.93 d | 1545.60 |
| ±SE | 19.70 | 10.81 | 288.01 |
| CV | 55.66 | 62.07 | 18.45 |
| min | 39.00 | 18.40 | 1216.56 |
| max | 150.00 | 88.40 | 1721.55 |
| 9 years old (n = 18) | | | |
| X | 90.83 | 99.63 cd | 1587.54 |
| ±SE | 8.04 | 4.41 | 117.58 |
| CV | 35.73 | 0.65 | 21.94 |
| min | 36.00 | 97.80 | 1147.70 |
| max | 142.00 | 100.00 | 2057.88 |

The differences between the values marked with the same symbols in each column are significant: c, d – $p < 0.05$

A number of studies have been conducted with Siberian sturgeon sperm. It is noteworthy that the sperm parameters found by us in the hybrid with the participation of the latter are better.

Glogowski et al. (2002) provide data on the characteristics of sperm in 3 Siberian sturgeons: 1 – concentration 0.217 x $10^9$ / ml (217 x $10^6$ / ml), motility – 80%; 2 – respectively 0.920 x $10^9$ / ml (920 x $10^6$ / ml), 45%; 3 – respectively 1,509 x $10^9$ / ml (1509 x $10^6$ / ml), 60%. The average sperm concentration was higher in all the groups of hybrids we studied, and the average values of motility generally approached or exceeded those established by the authors.

Sieczynski et al. (2012) obtained a value for total motility of 41.3% in the analysis of Siberian sturgeon sperm, which is lower than all values for motility in the different age groups, subject of our study. Sarosiek et al. (2004) also in Siberian sturgeon received a higher percentage of sperm motility (76%), at a sperm concentration of 0.64 x $10^9$ / ml (640 x $10^6$ / ml). The motility found by them is close to the values obtained by us in the 7-year-old hybrid, while the concentration of sperm in our study is significantly higher in all groups studied. Psenicka et al. (2008) found a concentration of 0.41 x $10^9$ / ml in Siberian sturgeon, which is more than four times lower than the maximum average concentration in our study and more than three times lower than the corresponding minimum average concentration.

There are fewer sperm production studies in hybrids. Noveiri et al. (2019) found sperm total motility at bester 81.2%. This value is lower by 22.69% than the total motility of 9-year-old hybrids in our study and is higher than our results in 7- and 8-year-old fish, by 4.96 and 25.06%, respectively.

We did not find data in the available literature on the macroscopic and microscopic properties of sperm in a hybrid between Siberian and Russian sturgeon, as well as data related to the enzymatic activity of sperm. This is probably due to the lower presence of the hybrid in sturgeon aquaculture. According to Bronzi et al. (2019) the hybrid (Siberian x Russian sturgeon) occupies a small relative share of Sturgeon species for caviar production – 0.55%, while the share of Siberian sturgeon occupies 30.9%. At the same time, Sturgeon farming has very good potential for F1 hybrids, thanks to their earlier maturation and better growth rates (Ivchenko et al., 2017). The Siberian and Russian sturgeon hybrid is used for aquaculture in Kazakhstan (Iskhakova & Khulmanova, 2014). In different hybrid forms (Russian sturgeon x Siberian sturgeon; Siberian sturgeon x Russian sturgeon) economic characteristics (survival, growth rates) features of the different stages of development are studied, the reproductive characteristics between the Russian sturgeon and the hybrid forms are compared (Miburo et al., 2018). Many interesting studies have been conducted by Grunina et al. (2011) for the production of androgenetic offspring in Siberian sturgeon and androgenetic hybrids between Siberian and Russian sturgeon.

We did not find studies of motility in Siberian and Russian sturgeon hybrid in the available literature. Such studies are available in a number of Sturgeon species in aquaculture. In general, it is difficult to compare sperm counts in different Sturgeon species obtained under different conditions. In addition to species characteristics, reproductive characteristics are influenced by technologies in which fish are raised. Elhetawy et al. (2020) found that fertility of Russian sturgeon was better in those reared in cages in a study of Russian sturgeon reared in RAS and combined in RAS and cages.

Sarosiek et al. (2004) did not find significant differences in a comparative study of some sperm parameters (sperm concentration and motility) in Siberian sturgeon and Sterlet. The authors believe that there are individual differences between male individuals rather than between species.

Psenicka et al. (2008) also found no significant differences in the percentage of motile sperm in Siberian sturgeon and Sterlet. Psenicka et al. (2007) note that the sperm of different Sturgeon species are similar in appearance with minimal morphological differences.

Fedorov et al. (2017) found higher values of VCL (176 µm / s) in Sterlet (*A. ruthenus*) than the obtained by us. Horokhovatskyi et al. (2018) also indicated the following motility characteristics – VCL (154.6 µm / s), VSL (111 µm / s) and LIN (72.1%) in Sterlet.

Sieczynski et al. (2012) obtained VCL (121.9 µm / s), VSL (82.5 µm / s), VAP (97.6 µm / s), LIN (58.6%), STR (82.3%), ALH 10.4 µm), BCF (4.9 Hz) in the analysis Siberian sturgeon semen. VCL and BCF were higher only in the oldest group in our study. All other indicators of motility in the same age group have lower levels. Each of the indicators of 7 and 8 – year – old hybrids has lower values.

Table. 2. shows that the spermatozoa in the oldest individuals have higher values ($p < 0.05$) of VCL, VSL and VAP, as the differences with 7-year-old fish are respectively 3.7, 2.49, 3.22 times; and with 8-year-olds respectively 5.19, 3.32, 4.24 times. The differences in the above indicators are not significant between hybrids of 7 and 8 years of age. The differences in LIN and STR are not significant between the groups, with the highest values obtained in 8-year-old hybrids. The highest WOB value was reported in 7-year-old fish, and the differences between the studied groups were not significant. The highest value of ALH and BCF was obtained in 9-year-old fish, and the difference with 7- and 8-year-old fish in the first indicator was 1.73 ($p < 0.05$) and 2.08 times ($p < 0.05$), respectively; and in the second 1.38 and 1.27 ($p < 0.05$) times, respectively.

It can be stated that the VCL varies from 9.22 to 160.59 µm / s; VSL from 1.90 to 67.50 µm / s; VAP from 3.90 to 101.60 µm / s; LIN from 14.90 to 64.30%; STR from 49 to 79.90%; WOB from 30.50 to 81.50%; ALH from 2.0 to 6.50 µm; BCF from 1.80 to 7.90 Hz by summarizing the data on the hybrid (F1 Ab x Ag) sperm motility.

**Table 2. Sperm motility in hybrid semen**

| Parameters | Indicators | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | VCL, µm/s | VSL, µm/s | VAP, µm/s | LIN, % | STR, % | WOB, % | ALH, µm | BCF, Hz |
| 7 years old | | | | | | | | |
| X | 35.90c | 19.20c | 24.80c | 43.10 | 62.10 | 68.70 | 2.80c | 4.50c |
| **±SE** | 5.31 | 4.73 | 4.30 | 5.17 | 3.54 | 4.87 | 0.35 | 0.57 |
| CV | 27.63 | 76.08 | 31.85 | 24.79 | 14.42 | 9.40 | 24.93 | 48.17 |
| min | 21.60 | 8.90 | 14.70 | 35.20 | 56.30 | 62.50 | 2.30 | 2.20 |
| max | 44.90 | 44.90 | 35.70 | 61.80 | 77.80 | 79.50 | 3.90 | 7.90 |
| 8 years old | | | | | | | | |
| X | 25.80d | 14.47d | 18.83d | 47.73 | 68.90 | 64.43 | 2.33d | 4.90 |
| **±SE** | 6.85 | 6.11 | 5.55 | 6.68 | 4.56 | 6.29 | 0.449 | 0.74 |
| CV | 44.07 | 75.25 | 68.69 | 59.57 | 25.01 | 45.61 | 12.37 | 54.80 |
| min | 12.70 | 1.90 | 3.90 | 14.90 | 49.00 | 30.50 | 2.00 | 1.80 |
| max | 32.80 | 21.00 | 26.60 | 64.30 | 78.90 | 81.50 | 2.50 | 6.50 |
| 9 years old | | | | | | | | |
| X | 133.82cd | 47.97cd | 79.88cd | 36.09 | 59.65 | 59.91 | 4.85cd | 6.22c |
| **±SE** | 2.80 | 2.49 | 2.27 | 2.73 | 1.86 | 2.56 | 0.18 | 0.30 |
| CV | 9.22 | 19.46 | 11.93 | 21.30 | 9.29 | 11.68 | 17.13 | 8.51 |
| min | 115.92 | 35.87 | 64.84 | 25.97 | 52.43 | 49.53 | 3.59 | 4.86 |
| max | 160.59 | 67.50 | 101.60 | 51.89 | 71.40 | 72.68 | 6.50 | 7.04 |

* VCL – curvilinear velocity; VSL – straight line velocity; VAP – average path velocity; LIN – linearity; STR – straightness; WOB – wobble; ALH – amplitude of lateral head displacement; BCF – beat/cross frequency. The differences between the values marked with the same symbols in each column are significant: c, d – $p < 0.05$.

The relative proportion of rapid sperm in the hybrid semen varies from 0 to 96.90%; of medium from 1.0 to 46.74%; at slow from 0.28 to 75.30%; static from 0 to 84% (Table 3). The relative share of spermatozoa with non – progressive motility varies from 15.30 to 89.70%, and those with progressive motility – from 0.10 to 29.85%.

The oldest fish (9-year-olds) have the best values of rapid sperm. The rapid sperm have the highest percentage in this group, as the difference with 7- and 8-year-olds is 30 ($p < 0.05$) and 41.8 ($p < 0.05$) times, respectively. The slow and static ones in the 9-year-old hybrid are the smallest, as the differences with the 7-year-olds are 8.4 ($p < 0.05$) and 59.6 ($p < 0.05$) times, respectively, and with the 8-year-olds they are 9.3 ($p < 0.05$) and 92.4 times. ($p < 0.05$) times.

There was no significant difference in the relative share of medium sperm in the individual groups.

No significant difference was obtained between the individual groups in the relative share of spermatozoa with non-progressive motility, as the average values of the indicator vary from 61.87 (8-year-old) to 78.75% (9-year-old). The share of spermatozoa with progressive motility is highest in the oldest fish (9 years old), as the difference in the indicator with 7-year-olds is 2.7 times ($p < 0.05$), and with 8-year-olds 6.96 times ($p < 0.05$).

It can be stated by summarizing the data that in the hybrid (F1 *A. baerii* x *A. gueldenstaedtii*) the oldest fish had the best characteristics of sperm motility – the highest share of rapid, the lowest of slow and static; the highest percentage of spermatozoa with progressive motility.

The enzyme activity of semen was studied in all age groups, but in the group of 8-year-olds the results of only two samples were obtained, due to which it is impossible to do the statistical processing of the data. The results for the individual enzymes in this group are as follows: in water extract AP – 53.0 – 68.0 UI / L, GGT – 11.98-14.29 UI / L, CK – 39.31-56.92 UI / L, LDH – 219.42 – 268.29 UI / L; in triton X100 extract – AP – 28.0 – 64.0 UI / L, GGT – 10.64 – 31.10 UI / L, CK – 9.21 – 20.12 UI / L, LDH – 46.74 – 95.18 UI / L. It is noteworthy that in the 8-year-old hybrid the values obtained for AP, CK in both water and triton X100 extract were lower compared to 7- and 9-year-old fish (Table 4).

A comparative analysis of the enzyme activity of 7- and 9-year-old fish semen shows that there is a significant difference between the groups of AP, GGT, CK in the water extract, and only the LDH in the triton X100 extract. There are significantly higher levels of AP (7 times; $p < 0.05$) and GGT (3.2 times; $p < 0.05$) in the water extract in older fish, while in younger fish the values of CK are higher (2.3 times; $p < 0.05$). In LDH, the difference is also in favor of younger individuals, but it is not significant (1.5 times; $p > 0.05$).

Only AP levels are higher (over 30%) in 9-year-old fish in the triton X100 extract, and the difference is not

**Table 3. Sperm distribution according to the characteristics of their movement and velocity in the hybrid semen, %**

| Parameters | Indicators | | | | | |
|---|---|---|---|---|---|---|
| | Rapid | Medium | Slow | Static | Non-progressive motile | Progressive motile |
| 7 years old | | | | | | |
| X | 2.36c | 24.26 | 50.72c | 22.66c | 69.74 | 7.60c |
| **±SE** | 6.13 | 7.17 | 6.46 | 8,37 | 8.00 | 2.95 |
| CV | 40.82 | 67.70 | 42.79 | 153.01 | 44.73 | 136.23 |
| min | 1.10 | 1.30 | 13.60 | 1.40 | 15.30 | 0.70 |
| max | 3.70 | 46.60 | 68.00 | 84.00 | 89.70 | 25.90 |
| 8 years old | | | | | | |
| X | 1.70d | 7.17 | 56.00c | 35.13d | 61.87 | 3.00d |
| **±SE** | 7,91 | 7.32 | 8.34 | 10,81 | 10.33 | 3.81 |
| CV | 86.65 | 74.55 | 59.69 | 114.73 | 60.97 | 83.79 |
| min | 0 | 1.00 | 17.40 | 11.70 | 18.30 | 0.10 |
| max | 2.60 | 10.40 | 75.30 | 81.60 | 83.80 | 4.60 |
| 9 years old | | | | | | |
| X | 71.00cd | 22.61 | 6.01cd | 0.38cd | 78.75 | 20.87cd |
| **±SE** | 3.23 | 2.99 | 3.41 | 4.14 | 4.212 | 1.56 |
| CV | 22.43 | 54.28 | 105.50 | 177.92 | 7.73 | 27.47 |
| min | 45.50 | 2.82 | 0.28 | 0 | 69.74 | 10.70 |
| max | 96.90 | 46.74 | 21.95 | 2.19 | 89.30 | 29.85 |

The differences between the values marked with the same symbols in each column are significant: c, d – $p < 0.05$

**Table 4. Hybrid sperm enzyme activity in water and triton X 100 extract, UI/L**

| Parameters | Indicators* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Water extract | | | | Triton X100 | | | |
| | AP | GGT | CK | LDH | AP | GGT | CK | LDH |
| 7 years old (n = 5) | | | | | | | | |
| X | 117.40c | 6.40c | 1515.30c | 1224.20 | 409.60 | 8.54 | 113.68 | 1531.10c |
| **±SE** | 49.62 | 2.14 | 311.56 | 230.43 | 139.75 | 2.45 | 15.09 | 268.86 |
| CV | 175.30 | 67.24 | 106.52 | 95.74 | 175.66 | 114.14 | 59.81 | 91.22 |
| min | 7.00 | 2.80 | 107.40 | 262.70 | 30.00 | 1.14 | 21.05 | 98.40 |
| max | 485.00 | 13.40 | 4268.60 | 3245.90 | 1695.00 | 19.30 | 191.20 | 3267.90 |
| 9 years old (n = 18) | | | | | | | | |
| X | 821.28c | 20.16c | 660.28*c* | 823.72 | 586.56 | 8.15 | 88.61 | 523.83*c* |
| **±SE** | 26.51 | 1.13 | 164.21 | 121.45 | 73.65 | 1.29 | 7.95 | 141.70 |
| CV | 9.40 | 24.89 | 18.62 | 17.29 | 11.46 | 28.17 | 22.07 | 17.83 |
| min | 679.00 | 12.23 | 488.00 | 580.00 | 492.00 | 4.87 | 56.00 | 390.00 |
| max | 935.00 | 30.25 | 873.00 | 1050.00 | 724.00 | 12.87 | 121.00 | 720.00 |

*AP – alkaline phosphatase; GGT – gamma glutamyltransferase; CK – creatine kinase; LDH – lactate dehydrogenase. The differences between the values marked with the same symbols in each column are significant: c – $p < 0.05$

significant. The difference is in favor of younger fish, respectively 4.6%; 22.1% and 2.9 times for the other indicators (GGT, CK and LDH), and for the latter it is significant ($p < 0.05$).

Enzyme levels in semen are studied in different teleost fish species. Ciereszko and Dabrowski (1994) found AP – 8.76 IU / L for rainbow trout (Onchorhyncus mykiss) and 4.77 IU / L for lake whitefish (Coregonus clupeaformis). Analyzing the levels of LDH in the sperm plasma of rainbow, river trout and carp, Atanasov (1996) confirms the claims of other authors that due to the intracellular nature of LDH, it can be used as a marker for the integrity of sperm membranes. The author concludes that the destructive changes that occur in sperm during sperm storage cause an increase in intracellular LDH to sperm plasma.

According to Huang et al. (2014) LDH and CK are essential for metabolic processes that provide energy for sperm survival, motility and fertility.

Piros et al. (2002) in a study of sperm quality obtained: for LDH 278.4 and 400.9 IU / L in Siberian sturgeon, 71.0 and 761.7 IU / L in Sterlet; for AP, 1.0 – 1.21 IU / L, and 0.90 and 1.85 IU / L, respectively. In our study in the hybrid, the lowest values for LDH were obtained in 9 – year – old individuals, respectively in water extract 823.72 IU / L, and in triton X100 extract – 523.83 IU / L. The values in water extract are 2 times higher, and in triton X100 extract by 23.5% higher than the highest obtained by the authors in Siberian sturgeon, which participates as a maternal form in obtaining the hybrid subject of our study. In the case of Sterlet, the highest value for LDH obtained by the authors was 761.7 IU / L, 7.5% lower than the value in water extract and 31% higher than the value obtained in triton X100 extract from our study in 9-year hybrids. The differences in AP values are huge. The lowest value reported in our study is 117.4 IU / L in water extract in 7-year-old hybrids. The highest value reported by the authors in Siberian sturgeon is 1.21 IU / L, 97 times lower than that obtained by us.

## Conclusions

Hybrids aged 7-, 8- and 9 years (F1 Ab x Ag), grown in a super-intensive cage farm located in a large warm water dam, have good sperm production. The difference in age does not significantly affect the volume of the ejaculate. In terms of motility, the oldest individuals have the highest values ($p < 0.05$) of VCL, VSL, VAP, ALH and BCF, as well as the best ($p < 0.05$) characteristics of sperm motility – the highest share of rapid and those with progressive motility and the lowest of the slow and static.

Age affects the enzyme activity of semen, with higher levels of AP (7 times; $p < 0.05$) and GGT (3.2 times; $p < 0.05$) in the water extract of older fish.

The values of CK (2.3 times; $p < 0.05$) and LDH (1.5 times) are higher in younger fish, but the differences are not significant.

The levels of AP in 9-year-old fish, in the triton X100 extract are higher than in the other age groups, but the differences are not significant. The differences are in favor of younger fish for the other indicators (GGT, CK and LDH), respectively 4.6%; 22.1% and 2.9 times, and for the latter it is significant ($p < 0.05$).

## References

**Akimochkina, T.I.** (2010). Cytological features of sperm of valuable fish species of the Volga-Caspian basin and their change depending on the conditions of cryopreservation. *PhD Thesis*, 153, (Ru).

**Alavi, S.M.H., Hatef, A., Mylonas, C., Gela, D., Papadaki, M., Rodina, M., Kaspar, V., Psenicka, M., Podhorec, P. & Linhart, O.** (2012). Sperm characteristics and androgens in Acipenser ruthenus after induction of spermiation by carp pituitary extract or GnRHa implants. *Fish Physiol Biochem, 38*, 1655–1666.

**Aramli, M.S., Kalbassi, M.R. & Nazari, R.M.** (2013). Blood and seminal plasma enzyme values of Persian sturgeon, Acipenser persicus (Chordata: Osteichthyes). *Italian Journal of Zoology*, *80 (4),* 490-493. DOI: https://doi.org/10.1080/11250003.2013.859309

**Atanasov, V.** (1996). Study of the effect of newly developed protective media for fish (*Salmo trutta m. fario* L., *Salmo gairdnerii irideus* G. и *Cyprinus carpio* L.) semen on the bioenergetic metabolism of gametes and their fertility. Dissertation, Trakia University, Stara Zagora, Bulgaria, (Bg).

**Bloesch, J.J., Jones, T., Reinartz, R. & Striebel, B.** (2006). An action plan for the conservation of sturgeons (Acipenseridae) in the Danube River Basin. *Österreichische Wasser- und Abfallwirtschaft, 58, 5-6*

**Bronzi, P., Chebanov, M., Michaels, J.T., Wei, Q., Rosenthal, H. & Gessner, J.** (2019). Sturgeon meat and caviar production: Global update 2017. *Journal of Applied Ichthyology*, *35(1),* 257-266. DOI: https://doi.org/10.1111/jai.13870.

**Ciereszko, A. & Dabrowski, K.** (1994). Relationship between biochemical constituents of fish semen and fertility: The effect of shortterm storage. *Fish Physiology and Biochemistry*, *12(5),* 357–367.

**Dzyuba, B., Cosson, J., Dzyuba, V., Fedorov, P., Bondarenko, O., Rodina, M., Linhart, O., Shelton, W. & Boryshpolets, S.** (2017). Sperm maturation in sturgeon (Actinopterygii, Acipenseriformes): A review. *Theriogenology*, *97*, 134-138.

**Elhetawy, A. I. G., Vasilyeva, L. M., Lotfy, A. M., Emelianova, N., Abdel-Rahim, M. M., Helal, A. M. & Sudakova, N. V.** (2020). Effects of the rearing system of the Russian sturgeon (*Acipenser gueldenstaedtii*) on growth, maturity, and the quality of produced caviar. *AACL Bioflux*, *13(6),* 3798-3809.

**Fedorov, P., Grabic, R., Fedorova, G., Cosson, J., Boryshpolets, S. & Dzyuba, B**. (2017). Development and application of LC/HRPS for quantification of adenine nucleotides, creatine phosphate, and creatine in sturgeon spermatozoa. *Czech Journal of Animal Science*, *62(2),* 67-74.

**Glogowski, J., Kolman, R., Mirosław, S., Horváth, A., Urbányi, B., Sieczyński, P., Rzemieniecki, A., Domagała, J., Demianowicz, W., Kowalski R. & Ciereszko, A.** (2002). Fertilization rate of Siberian sturgeon (Acipenser baeri, Brandt) milt cryopreserved with methanol. *Aquaculture*, *211(1),* 367-373.

**Grunina, A.S., Rekubratsky, A.V., Tsvetkova, L.I., Barmintseva, A.E., Vasilieva, E.D., Kovalev, K.V. & Poluektova, O. G.** (2011). Dyspermic androgenesis in sturgeon fish using cryopreserved sperm: obtaining androgenetic offspring of Siberian sturgeon and androgenetic hybrids between Siberian and Russian sturgeons. *Ontogenesis*, *42(2),* 133–145, (Ru).

**Hajirezaee, S., Amiri, B. M. & Mirvaghefi, A.** (2010). Fish milt quality and major factors influencing the milt quality parameters: *A review. African Journal of Biotechnology*, *9 (54),* 9148-9154.

**Horokhovatskyi, Y., Dietrich, M., Lebeda, I., Fedorov, P., Rodina, M. & Dzyuba, B.** (2018). Cryopreservation effects on a viable sperm sterlet (Acipenser ruthenus) subpopulation obtained by a Percoll density gradient method. *Plos One, 13(8)*, e0202514. https://doi.org/10.1371/journal.pone.0202514.

**Huang, X.R., Zhuang, P., Zhang, L., Liu, J.Y., Zhang, T., Feng, G. & Zhao, F.** (2014). Effect of cryopreservation on the enzyme activity of Russian sturgeon (Acipenser gueldenstaedtii Brandt & Ratzeburg, 1833) semen. *Journal of Applied Ichthyology*, *30(6),* 1585-1589.

**Iskhakova, G.O. & Khulmanova, G.A.** (2014). An experience of breeding the hybrid between Siberian and Russian sturgeon in Kazakhstan. *Research, Results, 1(5),* 15-19, (Ru).

**Ivchenko, A.A., Astafyeva, S.S. & Sudakova, N.V.** (2017). Morphophysiological characteristics of gonad development of sturgeons in cage conditions in the Volga delta. *Proceedings Of The Russian State Hydrometeorological University*, *46*, 169-173. ISSN 2074-2762, (Ru).

**Kowalski, R.K. & Cejko, B.I.** (2019). Sperm quality in fish: Determinants and affecting factors. *Theriogenology*, *135*, 94-108, DOI: https://doi.org/10.1016/j.theriogenology.2019.06.009

**Kuderskiy, L. A.** (2015). Researches on ichthyology, fishery and related subjects. Acclimatization of fishes in reservoirs of Russia. *Collection of scientific works of FGBNU of "GOSNIORKH"*. Sankt-Peterburg, 343, (Ru).

**Miburo, Z., Kokoza, A.A. & Alymov, Y.V.** (2018). Polyfunctional assessment of certain objects of Sturgeon fish (Acipenseridae) cultivated under conditions of aquaculture farms of lower Volga. *Problems of Fisheries*, *19(2),* 217-225, (Ru).

**MZH** (2021). Order of banning sturgeon fishing Nr RD09-1029/161220. https://www.mzh.government.bg/media/filer_public/2020/12/23/zapoved_esetri_1enteyc.pdf

**Noveiri, B., Bahmani, M., Yazdani Sadati, M.A. & Akbarzadeh, A.** (2019). Effects of seminal plasma ionic content, pH and osmolality on spermatozoa motility in bester (Female Huso huso × Male Acipenser ruthenus) sturgeon. *Iranian Journal of Fisheries Sciences*, *18(3),* 395-404.

**Piros, B., Glogowski, J., Kolman, R., Rzemieniecki, A., Domagala, J., Horvath, A. & Urbanyi, B., Ciereszko, A.** (2002). Biochemical characterization of Siberian sturgeon, Acipenser baeri and starlet, Acipenser ruthenus, seminal plasma and spermatozoa. *Fish Physiology and Biochemistry*, *26(3),* 289–295.

**Psenicka, M., Alavi, S., M. H., Rodina, M., Gela, D., Nebesarova, J. & Linhart, O.** (2007). Morphology andultrastructure of Siberian sturgeon (Acipenser baerii) spermatozoa using scanning and trans-mission electron microscopy. *Biology of the Cell*, *99(2)*, 103-15.

**Psenicka, M., Alavi, S., Rodina, M., Cicova, Z., Gela, D., Cosson, J., Nebesárová, J. & Linhart, O.** (2008). Morphology, chemical contents and physiology of chondrostean fish sperm: A comparative study between Siberian sturgeon (Acipenser baerii) and sterlet (Acipenser ruthenus). *Journal of Applied Ichthyology*, *24(4),* 371 – 377. DOI: https://doi.org/10.1111/j.1439-0426.2008.01139.x

**Sadeghi, A., Imanpoor, M.R., Shahriari, R., Khalili, M. & Abedi, M.** (2013). Cryopreservation of stellate (acipenser stellatus) sperm: Effect of different concentrations of DMSO and dilution rates on sperm mobility and motility duration after long-term storage. *Global Veterinaria*, *10 (1),* 26-30.

**Sadeghi, A., Pourmozaffar, S. & Gozari, M.** (2020). Comparison of some spermatological and biochemical parameters between Huso huso and Acipenser stellatus. *Journal of Animal Environment*, *12(2),* 117-122.

**Sarosiek, B., Ciereszko, A., Rzemieniecki, A., Domaga, J. & Glogowski, J.** (2004b). The influence of semen cryopreservation on the release of some enzymes from Siberian sturgeon (Acipenser baerii) and Sterlet (Acipenser ruthenus) spermatozoa. *Archives of Polish Fisheries*, *12(1),* 13-21.

**Shaluei, F., Sadeghi, A. & Zadmajid, V.** (2017). Cryopreservation of Persian sturgeon (Acipenser persicus) sperm: Effects of cryoprotectants, antioxidant, membrane stabilizer, equilibration time and dilution ratio on sperm motility and fertility. *Aquaculture Research*, *48 (3),* 1031-1040.

**Sieczyński, P., Glogowski, J., Cejko, B. & Grygoruk, C.** (2012). Characteristics of Siberian sturgeon and Sterlet sperm motility parameters compared using CASA. *Archives of Polish Fisheries*, *20(2),* 137-143. DOI: https://doi.org/10.2478/v10086-012-0016-0.