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RESEARCH ARTICLE

Characteristics of Siberian sturgeon and sterlet sperm motility parameters compared using CASA

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Abstract. Computer Assisted Sperm Analysis (CASA) was used to evaluate and compare the sperm motility parameters of Siberian sturgeon, *Acipenser baerii* Brandt (n=15), and sterlet, *Acipenser ruthenus* L. (n=15). Analysis indicated a higher percentage of motile sperm (MOT) in the sterlet semen (44.8%) than in that of the Siberian sturgeon (41.3%). The sperm of the two species studied were similar with regard to velocity, and similarities were also noted with regard to average path velocity VAP (97.6 and 102.4 μm s^{-1}), curvilinear velocity VCL (121.9 and 119.9 μm s^{-1}), and straight line velocity VSL (82.5 and 86.8 μm s^{-1}). Using the CASA system permitted making precise evaluations of the sperm motility of the two fish species studied, and thus also permitted determining the potential suitability of the semen for use in sturgeon reproduction.

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Introduction

Issues of gamete quality are especially important in the study of reproductive biology. Evaluating their biological quality is significant particularly with regard to culture programs for commercially important species such as carp, *Cyprinis carpi*o L., rainbow trout, *Oncorhynchus mykiss* Walbaum, and sturgeon species. Nearly all species of sturgeon are currently threatened, and many of them have very limited ranges of occurrence (Williot et al. 1997). Sturgeon fishes are an essential element in the maintenance of biological equilibrium in aquatic systems. They are targeted by fisheries for their delicious meat and are particularly valued for their eggs, which are used for caviar production (Gundersen and Pearson 1992, Mims and Shelton 1998). Artificially reproducing these fish and culturing them under controlled conditions might be a successful method for preventing populations from becoming extinct.

The most significant aspect of male reproductive biology is milt quality, which includes the ability of the sperm to fertilize eggs. Thus, positive, significant correlations have been confirmed repeatedly between sperm motility and the percentage of fertilized

eggs (Moccia and Munkittrick 1987), and the percentage of motile sperm is one of the basic measures used to determine milt quality. Until the 1990s sperm motility was evaluated subjectively under a light microscope. As computer technology developed, Computer Assisted Semen Analysis (CASA) systems began to be used to analyze sperm motility (Kime et al. 1996, Ravinder et al. 1997, Dietrich et al. 2005). CASA was recognized by international expert bodies as a reliable research and diagnostic tool for determining the fertility of male gametes (Eshre 1998). Examples of the effective application of CASA include studies by Kime and Tveiten (2002), who used it to evaluate fresh semen of the spotted wolffish, *Anarhichas minor* Olafsen. They concluded that of the of the 14 parameters measured with the CASA system, the six most vulnerable to changes in pH and activating fluid osmolality over the period of sperm activity were curvilinear velocity (VCL), straight line velocity (VSL), average path velocity (VAP), beat cross frequency (BCF), amplitude of lateral head displacement (ALH), and percentage of motile sperm (MOT). Consequently, this method can be considered the most useful for evaluating sperm motility. The other parameters were of a low diagnostic value. The use of CASA in toxicological studies, such as those focusing on the impact of xenobiotics or heavy metals on the motility parameters of commercially important fish species, attest to its wide range of applications (Cosson et al. 2000, Dietrich et al. 2010, Jarmołowicz et al. 2010).

Many studies focusing on sperm motility in other fish species and higher vertebrates (mammals) have proven that there is a significant, positive correlation between the values of CASA parameters and the fertilization rates (Gage et al. 2004). Demonstrating there are such strong co-dependencies in sturgeon would permit predicting fertilization success without having to perform costly and long-term biological trials. Very few studies in the literature focus on the application of CASA for sturgeon fish, and the majority of these refer to the use of it to evaluate milt quality after freezing (Ciereszko et al. 1996, Glogowski et al. 2002). The aim of the current study was to evaluate and compare sperm motility parameters in fresh semen from Siberian sturgeon, *Acipenser baerii* Brandt, and sterlet, *Acipenser ruthenus* L., using Computer Assisted Semen Analysis (CASA).

Materials and methods

Origin of males

The milt was obtained from males in December and January from two available sources: the Department of Sturgeon Fish Breeding in Pieczarki, Inland Fisheries Institute in Olsztyn and the Experimental Center of the Department of General Zoology, University of Szczecin where fish are cultured in the discharge waters of the Lower Oder Power Plant. Siberian sturgeon males from Dgał were aged 7+, body weight 7-10.5 kg, and total length (TL) of approximately 110 cm, while the sterlet males were aged 2-3 years with body weights of 0.39-1.25 kg and total length of approximately 47-60 cm. The Siberian sturgeon males were cultured from fertilization and held in tanks that were part of a closed recirculating system for three years, when they were transferred to earthen ponds. In December, two weeks prior to stimulation, both sturgeon species were moved to tanks with thermoregulation and controlled environmental parameters. During this time the water temperature was gradually increased from 7 to 12°C. The Siberian sturgeon males from the Lower Oder center were aged 3+, with body weights of 3-3.5 kg and total lengths of TL approximately 60-65 cm; however, there is no data regarding sterlet males. It is known only that the sterlet males were aged 2+ to 5+. Both sturgeon species were cultured until January in a pond located in warm canal waters at a temperature of 17°C.

Hormonal stimulation and spawners manipulation

Siberian sturgeon and sterlet males from the Dgał hatchery were hormonally stimulated with one intraperitoneal injection of sturgeon pituitary at a dose of 2 mg kg^{-1} body weight, while the fish from the

experimental center at Lower Oder were administered Ovopel [(D-Ala^6, Pro^{9}NEt) – mGnRH + metoclopramide] at a dose of 1 granule kg^{-1} m.c. One granule of Ovopel contains 18-20 μg mammalian analogue GnRHa and 8-10 mg metoclopramide (Horvath et al. 1997). The maturity state of the males was evaluated based on their external characteristics such as a spawning rash on the head and the appearance of the genital papilla. Twenty-four hours after hormonal stimulation, the males were anesthetized with 0.2% Propiscin in 28 ml l^{-1} water. Then the milt was collected with a 4 mm diameter catheter fitted with a 50 ml syringe (Kolman et al. 1998). The milt from individual males was collected into sterile containers and then placed on ice (2-4°C). After collection, the semen was transported to the laboratory where it was analyzed within 20 minutes. The total volume of milt obtained from Siberian sturgeon was 73 ± 58 ml at a concentration of 0.58 ± 0.37 mld ml^{-1}, while from sterlet it was 18 ± 13 ml and 0.46 ± 0.25 mld ml^{-1}, respectively.

Evaluation of Siberian sturgeon and sterlet sperm motility after collection and before activation

Before activating the sperm, the milt samples were checked to ensure they had not been contaminated with urine during collection. The subjective light microscope method at a magnification of 400x was used to assess sperm motility before activation. One μl of unactivated milt was placed on a glass slide and the number of motile sperm was estimated to the nearest ± 1%.

Evaluation of Siberian sturgeon and sterlet sperm motility parameters determined with CASA

Selected Siberian sturgeon and sterlet sperm motility parameters were determined using the computerized Hobson Sperm Tracker (HST) (Sence & Vision Electronic System Ltd. Sheffield, UK.). For the needs of the study and in order for HST to recognize the sturgeon sperm, the following settings were used: Thresholds +13/-12; Filterweightings 1:2; Filterweightings 2:2; Filterweightings 3:0; Filterweightings 4:0. The analysis time was set at 30 seconds. During sperm motility analysis the ambient temperature was lowered to 15°C, and the semen was held on ice (2-4°C). The temperature of the microscope table on which the tests were conducted was about 15°C. Sperm motility was activated with Jähnichen's buffer at a ratio of 1:25 (100 μl milt mixed with 2500 μl of activating fluid) composed of 10 mM Tris-HCl, 20 mM NaCl, 2 mM CaCl2, and pH 8.5 (Jähnichen et al. 1999), and the preparation was mixed as quickly as possible. Measurements were performed within approximately 5-10 seconds of initiating sperm motility. The analyses were performed on 4 μl of the milt mixture and activating buffer that was covered with a cover slide under controlled pressure. The sperm in the analyzed field were registered for 30 seconds. The motile sperm which marked paths were analyzed with CASA using a series of algorithms. The primary parameters were analyzed, i.e., percentage of motile sperm (MOT, %), average path velocity (VAP, μm s^{-1}), curvilinear velocity (VCL, μm s^{-1}), straight line velocity of sperm (VSL, μm s^{-1}), mean angular displacement (MAD, °), amplitude of lateral head displacement (ALH, μm), beat cross frequency (BCF, Hz). The following secondary parameters were also analyzed, i.e., linearity (LIN, %), path straightness (STR, %), and momentum (MOM, m s^{-1}). The milt of each specimen was activated twice so it could be observed two times. The means of CASA results were used in the statistical analysis.

Statistical analysis

Differences in sperm motility between the sterlet and Siberian sturgeon were assessed by independent Student's t-test ($P < 0.05$). The Kolmogorov-Smirnov test was applied to assess a normal distribution. Statistical analysis was performed with STATISTICA 8.0 PL and Excel 2007.

Results

Siberian sturgeon and sterlet sperm motility after collection and before activation

The values of Siberian sturgeon and sterlet sperm motility after collection without activation are presented in Table 1. Motility of single sperm in the milt of both species before activation was about 2%.

Siberian sturgeon and sterlet sperm motility parameters determined with CASA

CASA indicated a greater share of motile sperm in the sterlet semen (44.8%) than in the Siberian sturgeon semen (41.3%), but this difference was not significant (Table 1). The sperm of both species examined was similar with regard to velocity, and these similarities also occurred in the values of VAP (97.6 and 102.4 μm s^{-1}), VCL (121.9 and 119.9 μm s^{-1}), and VSL (82.5 and 86.8 μm s^{-1}). No statistically significant differences were observed between the Siberian sturgeon and sterlet sperm with regard to the values of ALH, BCF, LIN, STR, and MOM. Only with regard to MAD values was a statistically significant difference confirmed between the sperm of Siberian sturgeon and sterlet (Table 1).

Discussion

The results of the current study indicate that using CASA permitted precisely evaluating sperm motility in the two fish species studied, which allows for determining the potential suitability of the semen for sturgeon fish reproduction. The subjective evaluation of sperm motility, which is the basic parameter of standard semen quality tests, is frequently the only method applied in reproductive studies. Many authors have demonstrated, however, that subjective evaluation of sperm motility is not very valuable in determining fertility or predicting the results of fertilization (Aitken et al. 1982, Bartoov et al. 1993). The evaluations of the same semen performed by experienced analysts using the subjective method differ substantially. These differences are particularly significant in the evaluation of motility parameters and are as high as from 30 to 60% (Amann 1989, Barros et al. 1973, Budworth et al. 1988). This can be

Table 1
Indicators of sperm motility in fresh semen from Siberian sturgeon (*Acipenser baerii*) and sterlet (*Acipenser ruthenus*). Arithmetic mean (M), standard deviation (SD), standard error of the mean (SEM), minimum value (min), maximum value (max). Groups with different letter indexes in the same row are statistically significantly different ($P < 0.05$)

	Siberian sturgeon (n=15)					Sterlet (n=15)				
Parameter	M	SD	SEM	min	max	M	SD	SEM	min	max
Sperm motility without activation (%)	2.4	2.5	0.7	0	10	1.4	1.7	0.7	0	10
Percentage of motile sperm MOT (%)	41.3	14.1	2.6	27	78	44.8	17.5	2.9	24	71
Average path velocity VAP (μm s^{-1})	97.6	14.0	3.6	77.7	124.8	102.4	12.0	3.1	80.2	118.9
Curvilinear velocity VCL (μm s^{-1})	121.9	17.3	4.5	92.6	149.4	119.9	19.1	4.9	94.9	158.2
Straight line velocity VSL (μm s^{-1})	82.5	14.3	3.7	60.3	103.3	86.8	9.3	2.4	71.1	98.2
Mean angular displacement MAD (°)	70.7[a]	19.1	4.9	41.1	98.8	33.5[b]	10.4	2.7	22.8	62.8
Amplitude of lateral head displacement ALH (μm)	10.4	2.7	0.7	7.0	16.1	12.8	3.4	0.9	7.4	18.3
Beat cross frequency BCF (Hz)	4.9	0.8	0.2	3.7	6.4	3.7	1.0	0.3	2.4	6.0
Linearity LIN (%)	58.6	11.0	2.8	40.6	83.2	55.1	6.2	1.6	47.0	70.4
Straightness STR (%)	82.3	9.9	2.6	65.4	97.0	72.5	11.1	2.9	57.7	92.8
Momentum MOM (m s^{-1})	4591	502	238	2586	6502	3955	904	307	1177	5235

explained by many causes including variable ejaculate quality, the lack of a determined dependence of the various parameters on fertilization capabilities, and the descriptive character of the tests that are subject to substantial fluctuation and interpretation errors (Bostofte et al. 1982, Jensen et al. 1997). In comparison to subjective methods that are loaded with substantial error and are limited to identifying the fraction of motile sperm, CASA permits taking objective measurements of approximately fifteen motility parameters that are significant for successful spawn fertilization (Gage et al. 2004). This is evidenced by the positive correlation between the percentage of motile sperm and fertilization in fish (Wilson-Leedy and Ingermann 2007).

The aim of evaluating sperm motility after collection and before activation was to determine if the semen had been contaminated with urine during collection. In most fish species, the sperm in the testes and vasa deferentia are immobile, and activation only occurs when the milt comes into contact with water, urine, or activating fluid. The duration of sperm motility depends on the species, and in sturgeons it is several hours (Toth et al. 1997), several minutes in carp and pike, *Esox lucius* L., and several seconds in Atlantic salmon, *Salmo salar* L., and rainbow trout (Kołdras and Bieniarz 1995). Sperm activation initiated by urine has been described in wels catfish, *Silurus glanis* L., carp, turbot, *Scophthalmus maximus* (L.), tilapia, *Oreochromis mossambicus* (Peters), and in tench, *Tinca tinca* (L.) (Rurangwa et al. 2004). In many instances the cause of contamination is the close proximity of the vas deferens to the ureter. Significant urine contamination can lower milt quality, which is observed in carp, wels catfish, and turbot (Dreanno et al. 1998). There are also fish species, such as the spotted wolffish, the sperm of which is active for several hours without activation, in contrast to most teleosts, and in marine waters they become immobile (Kime and Tveiten 2002). In the current study, a small percentage of sperm were active immediately following semen collection and before activation. It is not yet known if this phenomenon, which has been noted repeatedly, is normal physiology in the reproductive biology of male sturgeons or if it is the result of milt contamination with water or urine (Glogowski et al. 2002).

CASA indicated that the average path velocity (VAP) and straight line velocity (VSL) of sperm in fresh sterlet semen were higher in comparison to those of Siberian sturgeon sperm. Glogowski et al. (2004) also reported higher mean values for VAP and VSL in sterlet semen of 133.3 $\mu m\ s^{-1}$ and 97.4 $\mu m\ s^{-1}$, respectively, in comparison to the semen of Siberian sturgeon at 115.9 $\mu m\ s^{-1}$ and 97.1 $\mu m\ s^{-1}$, respectively. The semen of Russian sturgeon, *Acipenser gueldenstaedtii* Brandt, was characterized by the highest mean VAP (144.9 $\mu m\ s^{-1}$) and VSL (123.9 $\mu m\ s^{-1}$). Ciereszko et al. (1996) determined the value of VSL in fresh semen of lake sturgeon, *Acipenser fulvescens* Rafinesque, to be 106.5 $\mu m\ s^{-1}$. There are studies which report positive dependencies between VSL values and the percentage of in vitro fertilization (Liu et al. 1991). Among all the sperm motility parameters evaluated by Rurangwa et al. (2004) using CASA, the parameters of VCL, VSL, and VAP were best correlated with the percentage of fertilization. Similar correlations between these CASA parameters and fertilization capabilities were confirmed in turbot (Dreanno et al. 1999), rainbow trout (Lahnsteiner 2000), carp (Linhart et al. 2000), and wels catfish (Rurangwa et al. 2001). In the present study, the mean values of VCL in Siberian sturgeon and sterlet were similar at 121.9 and 119.9 $\mu m\ s^{-1}$, respectively. Earlier studies indicated that mean VCL values were 137.4 $\mu m\ s^{-1}$ in Siberian sturgeon, 150.0 $\mu m\ s^{-1}$ in sterlet, and 159.5 $\mu m\ s^{-1}$ in Russian sturgeon (Glogowski et al. 2004). Ciereszko et al. (1996) reported much higher mean VCL values in lake sturgeon at 336.1 $\mu m\ s^{-1}$.

There is little information in the available literature regarding the MAD parameter and its link with the degree of fertilization. The current analysis indicated a statistically significantly higher mean value of MAD in the semen of Siberian sturgeon (70.7°) in comparison to that of the sperm of sterlet (33.5°). The study by Kime and Tveiten (2002) reported a MAD value in fresh spotted wolffish of 94°, which, according to the authors, is a very high mean in comparison to other teleost fish (30-40°).

In the current study, significant differences in the semen quality of individual males was observed in many sperm motility parameters. The differences noted in sperm motility could have stemmed from the different periods of semen collection, and from differences among individual specimens such as age, body weight, or overall physical condition or health of the fish. These suppositions could be confirmed by the high values of standard deviation among a given sturgeon species. Differences in sperm motility in individual Siberian sturgeon and sterlet males were confirmed in the studies by Billard (1986). Such fluctuations in semen quality within a given species have also been confirmed in carp, pike, and guppy, *Poecilia reticulata* Peters (Billard 1986).

Conclusions

- The low sperm motility after semen collection and before activation might indicate the collection method used on these sturgeon is appropriate.
- CASA permitted precisely determining sperm motility following activation, which, in turn, permitted evaluating the potential suitability of the semen for reproducing sturgeon fishes. The biological similarities between the characters of Siberian sturgeon and sterlet sperm motility were many, and the greatest of these was noted in the values of VAP, VCL, and VSL.
- Significant differences were detected in the individual sperm motility parameters among males from the same sturgeon species. The high values of standard deviation might indicate differing semen quality, which suggests there are possible differences in fertilization capabilities.

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Author contributions. P.S. ran the experiment, analyzed the results, and described them; J.G. provided supervision for the entirety of the experiment, B.I.C. described the results; C.G. performed the statistical analyses of the results.

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