

Effects of seminal plasma ionic content, pH and osmolality on spermatozoa motility in bester (Female *Huso huso* × Male *Acipenser ruthenus*) sturgeon

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Received: February 2017

Accepted: June 2017

Abstract

The ionic contents, pH and osmolality of seminal plasma and their relationships to sperm motility indices (sperm motility duration and percentage of motile sperm) were evaluated in bester. Semen was collected from six bester specimens and their seminal plasma was evaluated. The seminal plasma contained 54.5 ± 2.8 mM sodium, 22.33 ± 2.5 mM chloride, 7.1 ± 1.2 mM potassium, 3.55 ± 0.6 mM calcium and 0.58 ± 0.09 mM magnesium. The result shows that Na^+ , Cl^- and K^+ (54.5 ± 2.8 , 22.33 ± 2.5 and 7.1 ± 1.2 mM, respectively) were predominant ions in bester seminal plasma like other sturgeons. However the concentrations of K^+ , Ca^{2+} and Cl^- in bester seminal plasma were higher than those reported in beluga and sterlet as parental species. The mean pH and osmolality of seminal plasma were 8.09 ± 0.34 and 127.6 ± 20.8 mOsm kg^{-1} , respectively. A significant negative regression was observed between osmolality and percentage motility ($r = -0.893$).

Keywords: Bester, Seminal plasma, Motility, Ion, Osmotic pressure

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Introduction

Wild sturgeon populations have considerably declined during the last thirty years mainly due to overfishing and environmental degradation (IUCN, 2016; Prokopchuk *et al.*, 2016). Sturgeon aquaculture has been considered as a substitution activity to reduce fishing pressure on their natural resources on the one hand and for providing meat and caviar to cover the rising demands on the other (Andrei *et al.*, 2016). Among several candidates of sturgeons and their hybrid for aquaculture, bester sturgeon (*Huso huso* × *Acipenser ruthenus*) is one of the most promising species. It is an extremely successful viable and fertile sturgeon hybrid and has a good potential to be cultured in fresh and brackish waters, (Burtsev, 1997), mono and poly culture systems (Chebanov and Billard, 2001) with rapid growth rates and early maturation (Andrei *et al.*, 2016). It has also been artificially reproduced at least up to the third generation (Carmona *et al.*, 2009), for production of other sturgeon hybrids i.e. big bester (Burtsev, 1997) or for induction of gynogenesis in other sturgeon species (Fopp-Bayat *et al.*, 2007).

The evaluation of sperm motility indices have been regularly considered as a basic assessment to qualify spermatozoa and to choose the best broods for fertilization trials (Fauvel *et al.*, 2010). In sturgeons, the spermatozoa motility is mainly affected by changing the surrounding ionic contents, pH and osmolality (Alavi *et al.* 2012c; Dzyuba *et al.*, 2013). Given the unique characteristics of seminal

plasma to protect the spermatozoa before releasing into the water (Sadiqul Islam and Akhter, 2011), the assessment of these parameters and their relationships in intact semen samples could provide basic information to define sperm activation solutions in aquaculture activities or immobilization solutions for short and long term preservation (Dzyuba *et al.*, 2013).

The relationships of ionic contents, pH and osmolality of seminal plasma have been studied in different sturgeon species so far (Psenicka *et al.*, 2008; Alavi *et al.*, 2012c), but there is no previous report addressing these parameters in bester sturgeon. The objective of the study was to determine the ionic contents, pH and osmolality of seminal plasma and their relations to sperm motility indices in bester sturgeon.

Materials and methods

Brood fish

The bester sturgeon brood fish (mean body weight 8600 ± 2180 g and mean total length 121.5 ± 12.9 cm) were provided from International Sturgeon Research Institute (Rasht, Iran) during two breeding seasons (March 2015-2016). Male broods were kept separate from females in circular fiberglass tanks (4000 l) with constant water renewal (20 L min^{-1}).

Milt collection

Males were injected intramuscularly with a single dose of luteinizing hormone releasing hormone analogue (LHRH-A2), at a concentration of $3 \mu\text{g}$

kg⁻¹ of body weight (Nazari *et al.*, 2010). The semen from six males were collected from urogenital papilla by aspiration through catheter (5–7mm diameter) connected to a 50-ml plastic syringe (Alipour *et al.*, 2009). The genital area was carefully dried to avoid contamination of semen samples with urine or water.

Evaluation of semen pH and osmolality

Semen was initially centrifuged at 800 g for 5 min (Heraeus, Germany), followed by secondly centrifugation at 10000 g for 10 min (Eppendorf, Germany) (Psenicka *et al.*, 2008). The pH and osmolality of the supernatant were measured with laboratory pH meter (Sartorius, Germany) and a freezing point depression osmometer (Roebeling, Germany), respectively. The extra supernatants were stored at -20°C until ionic analysis.

Evaluation of sperm motility indices

Semen samples were diluted after adding 10 µl of fresh semen to 100 µl of water under a pre-focused microscope. The motility of spermatozoa was recorded within 15 seconds post activation using a CCD video camera (OCTAX, Spain). The first 25 successive frames of video records were considered to evaluate percentage of motile spermatozoa (Alavi *et al.*, 2012a). Total period of motility was

assessed using a sensitive laboratory chronometer (Hanhart, Germany) (±1 sec) until approximately more than 90% of the spermatozoa stopped their progressive movement.

Ionic content evaluation

To determine the concentrations of magnesium, chloride and calcium ions in seminal plasma, samples were subjected to spectrophotometric procedure (Unico, USA). Potassium and sodium ions were determined with an Ion Selective Electrodes (Caretium, Germany) method with special selective potassium and sodium electrodes.

Statistical analysis

Data were subjected to evaluation using SPSS 20.0 for Windows. All values were expressed as mean±SD. Correlations between measured parameters were performed using Pearson's correlation test. Differences were considered significant at $p < 0.05$.

Results

The ionic composition of seminal plasma and sperm motility indices are summarized in Table 1. Spermatozoa motility indices (duration and percentage) showed high variations among samples (Table 1). Sodium and chloride were predominant ions in the seminal plasma while magnesium ion was the minor constituent (Table 1).

Table 1: Descriptive statistics of pH, osmolality and ionic contents (Ca²⁺, K⁺, Na⁺, Mg²⁺, Cl⁻) of seminal plasma, sperm motility duration and percentage in bester sturgeon (n=6).

Parameter	Min	Max	Mean± SD
pH	7.51	8.68	8.09±0.34
Osmolality (mOsm kg ⁻¹)	91	157	127.6±20.8
Ca ²⁺ (mM)	3	4.8	3.55±0.6
K ⁺ (mM)	5.7	9.4	7.1±1.2
Na ⁺ (mM)	50	58	54.5±2.8
Mg ²⁺ (mM)	0.51	0.74	0.58±0.09
Cl ⁻ (mM)	19	26	22.33±2.5
Motility duration (s)	138	538	318±136
Motility percentage (%)	36.1	97.6	81.2±20.6

Correlations between ionic composition of seminal plasma and sperm motility indices are shown in Table 2. There was a significant negative relationship between seminal plasma osmolality and spermatozoa motility percentage

($p < 0.05$, $r = -0.893$). However, no significant correlations were observed between seminal plasma osmolality, pH and ionic composition of the seminal plasma with sperm motility duration ($p > 0.05$).

Table 2: Correlations between ionic compositions of seminal plasma and sperm motility indices in bester (*: $p < 0.05$, n=6).

	Osmolality	pH	Ca ²⁺	K ⁺	Na ⁺	Mg ²⁺	Cl ⁻	Motility duration
pH	-0.485							
Ca ²⁺	-0.637	0.711						
K ⁺	0.393	-0.217	-0.299					
Na ⁺	-0.66	-0.153	0.116	0.231				
Mg ²⁺	0.509	-0.403	-0.777	0.782	-0.121			
Cl ⁻	0.115	-0.453	0.195	0.409	0.558	-0.069		
Motility duration	-0.278	-0.701	-0.571	0.166	0.69	0.233	0.312	
Motility percentage	-0.893*	-0.47	0.235	0.128	0.131	-0.221	0.869	0.034

Osmolality (mOsm kg⁻¹); Ion concentrations (mM); Motility duration (seconds); Motility percentage (%)

Discussion

Evaluation of ionic contents, pH and osmolality of seminal plasma and their effects on spermatozoa motility, provide basic information to establish precise activation or immobilizing media both for aquaculture and conservation points of view (Sadiqu Islam and Akhter, 2011). Almost in all studied sturgeon species, the Na⁺, Cl⁻ and K⁺ ions are predominant in seminal plasma (Alavi *et al.*, 2012c), but they are lower than the concentrations observed in cyprinids and salmonids (Alavi and Cosson, 2006). The mean

concentrations of Na⁺, Cl⁻ and K⁺ ions in seminal plasma in bester were evaluated as 54.5±2.8, 22.33±2.5 and 7.1±1.2 (mM), respectively which showed the same predominance as in other sturgeons.

The comparison of mean concentration of K⁺ in seminal plasma of bester (7.1±1.2 mM) with other sturgeons showed that it was only lower than that of Chinese sturgeon (*A. sinensis*) (7.8 mM) (Alavi *et al.*, 2012c), but more than the maximum K⁺ concentrations reported in beluga (3.33 mM) (Baghfalaki *et al.*, 2009) and

sterlet (3.3 mM) (Alavi *et al.*, 2012a), as its parental species. It has been revealed that K^+ is a major inhibitor ion for sperm motility in *A. fulvescens* (Toth *et al.*, 1997), *Polyodon spathula* (Linhart *et al.*, 2002), *A. brevirostrum* and *Scaphirhynchus platyrhynchus* (Wayman 2003) and *Acipenser persicus* (Alavi *et al.*, 2004), but its effective inhibitory concentrations differ among sturgeon species (Alavi *et al.*, 2012b). In bester, neither correlations between K^+ concentration and sperm motility duration nor K^+ concentration and sperm motility percentage were significant. It has been suggested that sturgeon spermatozoa motilities probably has a dual control mechanisms by K^+ ion concentrations as well as osmolality of the seminal plasma (Alavi *et al.*, 2012b; Prokopchuk *et al.*, 2016). The mean Na^+ concentration in seminal plasma of bester (54.5 ± 2.8 mM) was lower than the studied sturgeon species except in beluga (84.9 ± 18.7 mM) (Baghfalaki *et al.*, 2009), Persian sturgeon (62.4 ± 6.8 mM) (Alavi and Cosson, 2006) and ship sturgeon (*Acipenser nudiiventris*) (65.2 ± 39.9 mM) (Shalouei *et al.*, 2008). These differences could be attributed to the origin of broods, different hormonal treatment, sampling methods and frequency of stripping among these studies (Psenicka *et al.*, 2008).

The concentration of Ca^{2+} in bester (3.55 ± 0.6 mM) showed more than those presented in beluga (0.9 mM) (Baghfalaki *et al.*, 2009) and sterlet (0.1-0.2 mM) (Psenicka *et al.*, 2008; Alavi *et al.*, 2011). It is revealed that the increase in the concentrations of

external cations (Ca^{2+} and Na^+) has an antagonistic effect on K^+ inhibitory action of sturgeon spermatozoa (Alavi *et al.*, 2012b). Alavi *et al.* (2011) showed that Ca^{2+} concentration at more than 2.5 mM made asymmetric flagellar beating, resulting in lower sperm motility and velocity in *A. ruthenus*. Such a phenomenon might be assumed in bester with Ca^{2+} concentration of 3.55 ± 0.6 mM.

Although Cl^- has been assessed as one of the most predominant ions among sturgeon seminal plasma, there is limited information about the effects of this ion on sperm motility indices (Alavi *et al.*, 2004). The comparison of Cl^- concentration in seminal plasma of bester (22.33 ± 2.5 mM) with other sturgeons, showed that it is only less than that of North American species such as paddlefish (*Polyodon spathula*) (38.7 mM), pallid sturgeon (*Scaphirhynchus albus*) (30.4 mM) and white sturgeon (*Acipenser transmontanus*) (35.7 mM) (Wayman 2003).

There is very scarce information about the relationship between Mg^{2+} content of seminal plasma and spermatozoa motility in sturgeon fishes. Negative correlation between Mg^{2+} and Cl^- has been already reported in Persian sturgeon (Alavi *et al.*, 2004). There were no significant relationships between Mg^{2+} concentration vs. sperm motility and percentage in bester (Table 2). In other families, Khara *et al.*, (2012), using series of Mg^{2+} concentration as activation solution in bighead carp (*Aristichthys nobilis*), showed that minimum content of Mg^{2+}

had the best effect on sperm motility duration (Khara *et al.*, 2012). Lahnsteiner (2014) reported the suppressive effect of Ca^{2+} and Mg^{2+} ions on sperm motility at concentrations of $\geq 1.5\text{mM}$, 10 seconds after activation, in perch (*Perca fluviatilis*). Such a phenomenon suggested a more complicated relationship among ions on fish sperm motility performances.

A high negative correlation was observed between seminal plasma osmolality and percentage of motility in bester ($p < 0.05$, $r = -0.893$). The motility duration did not show significant relations with osmolality. In case of osmolality, wide ranges of inter and intraspecific differences in sturgeons suggested that the mechanism of sperm motility initiation is diverse and therefore, osmotic pressure of the seminal plasma could not be considered as a basic biological factor to control sperm motility (Ingermann *et al.*, 2002; Alavi *et al.*, 2012b). The range of seminal plasma osmolality evaluated in bester ($91\text{-}157\text{ mOsm kg}^{-1}$), could be due to age and feeding of brood or water temperature (Alavi *et al.*, 2012a).

The alkaline condition of seminal plasma among sturgeons (7.5-8.5) has been mentioned before (Alavi *et al.*, 2012c). In this study, the mean evaluated semen pH was 8.09 ± 0.34 , which is lower than those reported for *A. ruthenus* (8.13 ± 0.19) (Psenicka *et al.*, 2008) as paternal, but in the range of those reported in beluga (7.75-8.5) (Baghfalaki *et al.*, 2009; Li *et al.*, 2010) as maternal species. Data showed that there are neither relationships between pH values of seminal plasma with

seminal plasma osmolality nor sperm motility duration and percentage in bester. Gallis *et al.*, (1991) reported that motility of sperm from Siberian sturgeon (*A. baeri*) is relatively insensitive to pH changes near physiological semen pH (approximately 8.1). Also no correlation was found between sperm motility and pH in *A. baeri* (Williot *et al.*, 2000). A negative relationship between pH and sperm motility percentage has been revealed in white sturgeon (*Acipenser transmontanus*) semen at $\text{pH} > 8.5$ (Ingermann *et al.*, 2002). Alavi and Cosson (2005) reported the increase of sperm motility duration and percentage of motile sperm from pH 6 up to pH 8, decreasing afterward up to pH 9 in *A. persicus* (Alavi and Cosson, 2005). It has been demonstrated that in a solution with 2.5 mM Ca^{2+} and pH 10, the acrosome reaction activated as a rate of 68%, followed by a significant decrease in motility percentage and sperm velocity in *A. ruthenus* (Psenicka *et al.*, 2011).

The present study shows that pH and osmotic pressure of seminal plasma in bester were at the range of other sturgeons. Sodium, chloride and potassium were predominant ions in seminal plasma of bester, respectively like other sturgeons. Also our findings revealed a negative correlation between seminal plasma osmolality and sperm motility percentage in this fish.

To our knowledge, no data are available on pH, ionic content and osmolality of seminal plasma with regards to spermatozoa motility indices in bester sturgeon. The results of this

study provided the basic information of ionic content in bester seminal plasma and their relationships with sperm motility indices. As the ionic content of extenders are critical for definition of activator or immobilization media, these data could be regarded for short and long term preservation extenders of bester semen. Meanwhile, the optimal concentration of each ion in bester seminal plasma with regard to motility indices needs to be evaluated in future. More additional studies are also needed to elucidate the synergistic effects between ions on bester sperm motility.

Acknowledgements

The authors would like to thank M. Pourdehghani for hormone administration, J. Jalilpour for data analysis and technical assistance of the Aquaculture department of International Sturgeon Research Institute (Rasht, Iran) for their great help on semen sampling.

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