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

Baradaran Noveiri S.¹,²; Noori A.¹*; Bahmani M.²; Yazdani Sadati M.A.²; Akbarzadeh A.¹

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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⁺, Ca²⁺ 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

1-Department of Fisheries, Faculty of Marine Sciences and Technologies, University of Hormozgan, P.O.Box: 3995, Bandar Abbas, Iran
2-International Sturgeon Research Institute, Agricultural Research, Education and Extension Organization (AREEO), P.O.Box:41635-3464, Rasht, Iran
*Corresponding author’s Email: nooryahmad@gmail.com
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, better 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 better (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 better 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 better sturgeon.

Materials and methods
Brood fish
The better 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 μg
kg⁻¹ of body weight (Nazari et al., 2010). The semen from six males were collected from urogenital papilla by aspiration through catheter (5–7 mm 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 (Roebling, 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 (Ca$^{2+}$, K$^+$, Na$^+$, Mg$^{2+}$, Cl$^-$) of seminal plasma, sperm motility duration and percentage in bester sturgeon (n=6).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Min</th>
<th>Max</th>
<th>Mean± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.51</td>
<td>8.68</td>
<td>8.09±0.34</td>
</tr>
<tr>
<td>Osmolality (mOsm kg$^{-1}$)</td>
<td>91</td>
<td>157</td>
<td>127.6±20.8</td>
</tr>
<tr>
<td>Ca$^{2+}$ (mM)</td>
<td>3</td>
<td>4.8</td>
<td>3.55±0.6</td>
</tr>
<tr>
<td>K$^+$ (mM)</td>
<td>5.7</td>
<td>9.4</td>
<td>7.1±1.2</td>
</tr>
<tr>
<td>Na$^+$ (mM)</td>
<td>50</td>
<td>58</td>
<td>54.5±2.8</td>
</tr>
<tr>
<td>Mg$^{2+}$ (mM)</td>
<td>0.51</td>
<td>0.74</td>
<td>0.58±0.09</td>
</tr>
<tr>
<td>Cl$^-$ (mM)</td>
<td>19</td>
<td>26</td>
<td>22.3±4.2</td>
</tr>
<tr>
<td>Motility duration (s)</td>
<td>138</td>
<td>538</td>
<td>318±136</td>
</tr>
<tr>
<td>Motility percentage (%)</td>
<td>36.1</td>
<td>97.6</td>
<td>81.2±20.6</td>
</tr>
</tbody>
</table>

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).

<table>
<thead>
<tr>
<th>Osmolality (mOsm kg$^{-1}$)</th>
<th>pH</th>
<th>Ca$^{2+}$</th>
<th>K$^+$</th>
<th>Na$^+$</th>
<th>Mg$^{2+}$</th>
<th>Cl$^-$</th>
<th>Motility duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>-0.485</td>
<td>-0.637</td>
<td>0.393</td>
<td>-0.66</td>
<td>0.509</td>
<td>0.115</td>
<td>-0.278</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td></td>
<td>-0.711</td>
<td>-0.217</td>
<td>-0.299</td>
<td>0.116</td>
<td>0.195</td>
<td>0.235</td>
</tr>
<tr>
<td>K$^+$</td>
<td></td>
<td></td>
<td>0.166</td>
<td>0.231</td>
<td>-0.777</td>
<td>0.409</td>
<td>0.058</td>
</tr>
<tr>
<td>Na$^+$</td>
<td></td>
<td></td>
<td></td>
<td>-0.453</td>
<td>-0.403</td>
<td>0.585</td>
<td>0.47</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.571</td>
<td>0.166</td>
<td>0.233</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.131</td>
<td>0.312</td>
</tr>
<tr>
<td>Motility duration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Motility percentage (%)</td>
<td>-0.893*</td>
<td>-0.47</td>
<td>0.235</td>
<td>0.128</td>
<td>0.131</td>
<td>-0.221</td>
<td>0.869</td>
</tr>
</tbody>
</table>

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 (Sadiqul 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, 22.3±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 platyrynchus* (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 nudiventris*) (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 ≥1.5mM, 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 (91-157 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 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
The 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.

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