Determination of some seminal plasma indices, sperm density and sperm motility in the Persian sturgeon, *Acipenser persicus*.

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Abstract: Some biological aspects of semen were investigated in the Persian sturgeon, *Acipenser persicus*, by determination of seminal plasma indices (ionic composition and osmolality), sperm density and their relationships with sperm motility. The osmolality of seminal plasma ranged from 42.00 to 111.00 mOsmol Kg⁻¹. The concentrations of Sodium (Na⁺), Potassium (K⁺), Chloride (Cl⁻), Calcium (Ca²⁺) and Magnesium (Mg²⁺) ions were 62.44±6.82, 6.92±0.88, 21.11±5.41, 0.79±0.03 and 0.52±0.03mM L⁻¹, respectively. The sperm density was 8.34±1.38×10⁹ spz/ml. The Sodium/Potassium and Calcium/Potassium ratios were 9.02 and 0.13,

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*This paper is dedicated to Mrs. Narges Ahmadian a specialist in Sperm Biology in Sturgeon who passed away two years ago.
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0.905, P<0.001) and Na⁺ - Cl⁻ (r² = 0.584, P<0.05). There were also no significant correlations between ionic composition and osmolality of the seminal plasma and sperm motility (P>0.05). No relationship was observed between sperm density and its motility at the concentrations tested (r² = 0.015, P>0.05). The ionic composition and osmolality of seminal plasma reveals an inter-species specific as well high secretory activity of sperm duct. The clear differences observed in A. persicus should be considered when cryopreservation methods for sperm are developed in Acipenseridae species.

Keywords: Acipenser persicus, ionic content, osmolality, seminal plasma, sperm density, sperm motility

Introduction

Acquisition of new knowledge about various aspects of semen biology and preservation is an important factor in controlling artificial fertilization procedures in fish culture and biological conservation of animal species (Tsvetkova et al., 1996; Alavi & Cosson, 2002). Semen is defined as seminal plasma plus spermatozoa. Seminal plasma has unique composition considering the presence of some substances supporting spermatozoa and of some substances reflecting the functions of the reproductive system and those of spermatozoa (Ciereszko et al., 2000).

Determination of semen quality, including sperm density, biochemistry of seminal plasma, sperm motility and their physiological inter-relationships is the main factor to be considered in creating an optimal medium for artificial fertilization and for cryopreservation (Suquet et al., 2000; Billard et al., 1995b; Perchec et al., 1995). Sperm density is low in sturgeon in comparison to the teleosts. But, motility of sperm of sturgeon is longer and reported from 1 min to more than 5 min (Billard et al., 1999). Sturgeon sperm those of like teleosts are immotile in the seminal plasma. The inhibition of motility is mainly due to osmolality in most species (Linhart et al., 1991; Cosson et al., 1999), but K⁺, for example plays a major role in salmonids (Schlenk & Kahmmann, 1938; Billard et al., 1995a) and in sturgeon (Gallis et al., 1991; Billard, 2000; Alavi, 2003; Alavi et al., 2002a,b, 2004).

Literature review on the subject reveals considerable intra- and inter-species variability in the ionic composition and osmolality of seminal plasma of fishes (Alavi & Cosson, 2005), mainly due to significant intra- and inter-specific
differences in testicular secretions (Billard et al., 1995a). The change in seminal plasma osmolality is in correlation with thinning (hydration) of sperm during spermiation (Morisawa et al., 1979).


The objectives of this study were: (a) to determine some biological aspects of semen of *A. persicus*, including seminal plasma indices (ionic composition and osmolality) and sperm density, and (b) to define if there was any correlation between the evaluated indices and sperm motility.

**Material and methods**

**Broodstock:**

These experiments were carried out in April 2002. The spawners of the Persian sturgeon, *Acipenser persicus* Borodin 1897, (119-159cm total length and 17-20.5Kg weight) were captured from the Sefied Roud River, Rasht, Iran. After transportation into the hatchery facilities of Dr. Beheshti Artificial Sturgeon Propagation and Rearing Center (BASPRC), fishes were kept in broodstock pond for a few days with water temperature 14-17°C and 8.2mg O₂ L⁻¹. The semen was collected by hand stripping, 24 hours after induction of spermiation by intramuscular injection of sturgeon pituitary extract (50-70 mg/Kg⁻¹ body weight) (Kohneshahri & Azari Takami, 1974). Contamination with water or urine was carefully prevented. Semen was immediately poured into glass tubes and transferred to the laboratory of Department of Physiology and Biochemistry at the International Sturgeon Research Institute (ISRI). Semen of the males was stored for one day at 4°C, until motility analysis began.
Sperm concentration:
Spermatozoa concentration was measured by counting, using Hemocytometer Counting Chamber, after decantation. The semen was diluted 1000 times, using 0.7% NaCl (Ciereszko & Dabrowski 1993).

Ionic composition and osmolality of seminal fluid:
Semen was immediately centrifuged (Heraeus, Sepatech, Labofuge 200, Germany), using a two step method, firstly at 500rpm for 2 min, and secondly at 3000 rpm for 10 min. The supernatant was freeze-dried and stored at -21°C, until use (Alavi, 2003). Osmolality of the seminal plasma was measured by an osmometer (Melting Point Osmometer Nr 961003, Roebling, Germany), using a freezing point depression. Determination of the ionic concentrations of seminal plasma was carried out at Dr. A. Fadaiee Medicine Laboratory, Rasht, Iran. Magnesium, Chloride, and Calcium were measured with colorimetric measurement using an Autoanalyser Technician (RA 1000, Technicon-Swords, Dublin, Ireland), while Potassium and Sodium were determined with a flamephotometer (Corning 480 Corning, Medfield, MA, USA).

Correlation between sperm density and sperm motility:
The correlation between sperm density, a quantitative parameter of semen, (as independent factor) and sperm motility (as dependent factor) was studied. The fresh sperm from six males were activated in saline solution containing 50mM NaCl, 20mM Tris-HCl, pH 8.0, in triplicate.

Relationship between seminal plasma indices and sperm motility:
The correlation between some qualitative parameters of seminal plasma, including ionic contents and osmolality (as independent factor) and sperm motility (as dependent factor), was studied. To activate sperm motility, milt was diluted; 1:50 in buffered freshwater containing 20 mM Tris-HCl, pH 8.0.
Motility analyses:

The motility analyses were immediately performed after dilution with water. Spermatozoa motility was evaluated by visual evaluation on the microscope glass slide (dilution 1:50 in the fertilization solution under 400X magnification), as the percentage of motile spermatozoa, at 5 sec after initiation of activation. The total period of motility was evaluated as the time (in seconds) needed for most spermatozoa (near to 100%) to reach immotility. All experiments were performed at room temperature (17-20°C).

Data analysis:

The reported data are the means of independent measurements. All mean value represent mean ±SEM. Mean values were compared by Independent sample T-test, in cases of ionic content, osmolality of seminal plasma, and total period of sperm motility. Statistical comparisons were made with the Mann-Whitney-U test in case of percentages. Regression fits were investigated by linear and non-linear regression models and ANOVA.

Results

The sperm density and seminal plasma indices:

The sperm density and some seminal plasma indices are shown in table 1. The average sperm density was 8.34±1.38×10⁹ spz/ml. The osmolality of seminal plasma ranged from 42.00 to 111.00, averaging 82.56±8.10 mOsmol Kg⁻¹. The concentrations of monovalent ions were higher than that of divalent ions (2 independent sample T-test, P<0.001). The Na⁺ had the highest concentration (62.44±6.82 mM) and the concentration of Cl⁻ (21.11±5.41 mM) was higher than K⁺ that of ions (6.92±0.88 mM) (Table 1). The concentrations of Ca²⁺ and Mg²⁺ were 0.79±0.03 and 0.52±0.03 mM, respectively. The Sodium/Potassium and Calcium/Potassium ratios were 9.02 and 0.13, respectively.
Correlations between seminal plasma indices:

The correlation between seminal plasma indices are shown in Table 2. The following significant positive correlations between seminal plasma parameters were found: osmolality -Cl⁻ ($r^2=0.492$, $P<0.05$), osmolality -Na⁺ ($r^2=0.905$, $P<0.001$), Na⁺ - Cl⁻ ($r^2=0.584$, $P<0.05$).

Table 1: Ionic composition (mM L⁻¹) and osmolality (mOsmol Kg⁻¹) of seminal plasma and sperm density ($\times10^9$ spz/ml) in the Persian sturgeon, *A. persicus*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm density</td>
<td>4.29</td>
<td>16.06</td>
<td>8.34</td>
<td>1.38</td>
</tr>
<tr>
<td>Osmolality</td>
<td>42.00</td>
<td>111.00</td>
<td>82.56</td>
<td>8.10</td>
</tr>
<tr>
<td>Na⁺</td>
<td>21.00</td>
<td>80.00</td>
<td>62.44</td>
<td>6.82</td>
</tr>
<tr>
<td>K⁺</td>
<td>3.70</td>
<td>11.80</td>
<td>6.92</td>
<td>0.88</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>2.00</td>
<td>43.00</td>
<td>21.11</td>
<td>5.47</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>0.41</td>
<td>0.62</td>
<td>0.52</td>
<td>0.03</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>0.66</td>
<td>0.91</td>
<td>0.79</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Table 2: Correlation coefficients and statistically significant correlations between seminal plasma indices (ANOVA, a: $P<0.001$; b: $P<0.05$).

<table>
<thead>
<tr>
<th></th>
<th>K⁺</th>
<th>Cl⁻</th>
<th>Mg²⁺</th>
<th>Ca²⁺</th>
<th>Osmolality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺ mM L⁻¹</td>
<td>0.2587</td>
<td>0.5844b</td>
<td>0.0186</td>
<td>0.0001</td>
<td>0.9053a</td>
</tr>
<tr>
<td>K⁺ mM L⁻¹</td>
<td>-------</td>
<td>0.2959</td>
<td>0.0140</td>
<td>0.0718</td>
<td></td>
</tr>
<tr>
<td>Mg²⁺ mM L⁻¹</td>
<td>-------</td>
<td>0.0992</td>
<td>-------</td>
<td>-------</td>
<td></td>
</tr>
<tr>
<td>Ca²⁺ mM L⁻¹</td>
<td>-------</td>
<td>0.0177</td>
<td>0.2099</td>
<td>-------</td>
<td></td>
</tr>
<tr>
<td>Osmolality</td>
<td>0.2022</td>
<td>0.4922b</td>
<td>0.0081</td>
<td>0.0171</td>
<td></td>
</tr>
<tr>
<td>(mOsmol Kg⁻¹)</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td></td>
</tr>
</tbody>
</table>
The correlation between sperm density and motility:
No relationship was observed between sperm density and motility both in the percentage of motile sperm and total period of sperm motility at the concentrations tested (Figure 1, \( r^2 = 0.015 \), \( n=7 \) from 6 males, ANOVA, \( P>0.05 \)).

![Graph showing the relationship between sperm density and motility characteristics](image)

Figure 1: The relationship between sperm density (\( \times 10^9 \) spz/ml) and sperm motility characteristics (the percentage of motile cells [\( \% \), ■] and total period of motility [Sec, •]) of *Acipenser persicus* (\( N=17 \) samples from 6 males, ANOVA, \( P>0.05 \)).

Correlation between seminal plasma indices and sperm motility:
Statistically significant correlations, and their regression functions, between seminal plasma quality and motility of spermatozoa are shown in Figure 2. The total duration of sperm motility and the percentage of motile spermatozoa to total spermatozoa were used as dependent, and seminal plasma parameters as independent variables. Neither percentage of motile cells nor total duration of sperm motility decreased when the osmolality of seminal plasma increased to more than 90 mOsmol Kg\(^{-1}\) (Figure 2a). Increase of Na\(^+\) concentration in seminal plasma (Figure 2b) increased the total duration of sperm motility. But, the percentage of motile spermatozoa decreased, when the Na\(^+\) concentration reached 70 mM or higher (Figure 2b). The highest percentage of motile spermatozoa was observed at
60mM Na⁺ level of the seminal plasma (Figure 2b). By increase in K⁺ concentration of the seminal plasma the percentage of motile sperm decreased but the total duration of motility was approximately constant (Figure 2c). Maximum total duration of motility of sperm was observed when the level of Ca²⁺ was about 0.8mM in the seminal plasma (Figure 2d). However, the percentage of motile cells was lowest in 0.75mM L⁻¹ concentration (Figure 2d). When increasing Cl⁻ concentration in the seminal plasma, the percentage of motile sperm increases, but the total duration of motility remains approximately constant (Figure 2e). The least total duration of sperm motility was observed, when the Mg²⁺ concentration was about 0.5mM L⁻¹ (Figure 2f). Also, the percentage of motile cells decreased, when the Mg²⁺ concentration decreased (Figure 2f). By increasing the Na⁺/ K⁺ ratio of seminal plasma neither percentage of motile spermatozoa nor the total duration of motility of sperm increased (Figure 2g). However, changes of that ratio decreased both the percentage and total duration of motility. The highest total duration of motility of spermatozoa was observed at 10.6 Ca²⁺/ K⁺ ratio in the seminal plasma (Figure 2h). By increasing the Ca²⁺/K⁺ ratio in the seminal plasma the percentage of motile sperm, also, increased (Figure 2h).

Figure 2: The relationships between seminal plasma indices [Osmolality, mOsmol Kg⁻¹ (panel a); Sodium, mM L⁻¹ (panel b); Potassium, mM L⁻¹ (panel c); Calcium, mM L⁻¹ (panel d); Chloride, mM L⁻¹ (panel e); Magnesium, mM L⁻¹ (panel f); Na⁺/K⁺ (panel g) and Ca²⁺/K⁺ (panel h)] and sperm motility characteristics (the percentage of motile cells [%, ■] and total period of motility [Sec, ●] in Acipenser persicus.
Figure 2:
Discussion

Sperm density and seminal plasma indices:

The minimum, maximum and mean of sperm concentration values for *A. persicus* obtained by Ginsburg (1968) (Min. 0.6 and Max. 1.5×10⁹ spz/ml), Ahmadian (2000) (Mean 0.64×10⁹ spz/ml) were lower than those recorded in the present study. The observed differences are thought to be correlated to numerous factors, including the size (Ginsburg, 1968), age (Suquet et al., 1998), and weight (Suquet et al., 1994) of the males, as well as to the gonadosomatic index (GSI), duration of broodstock participation in spawning (Ginsburg, 1968), environmental factors (Pohl-Branscheid & Holtz, 1990), sampling period (Suquet et al., 1992) and sampling methods (Billard et al., 1995a ; Suquet et al., 1992, 1994).

The osmolality and levels of Na⁺, Cl⁻, Mg²⁺, and Ca²⁺ of the seminal plasma of *A. persicus* are higher than those of other Acipenserids species (e.g., *A. baeri*, Gallis et al., 1991; *A. fulvecensce*, Toth et al., 1997) (Table 3). This may represent species-specific characteristics. The K⁺ level in *A. persicus* is higher than that in *A. baeri*, but is close to that of *A. fulvecensce* (Toth et al., 1997) (Table 3). The formation of the seminal plasma components (both inorganic and organic) is an active secretion process of the spermatic duct epithelium (Marshall, 1989 ; Lahnsteiner et al., 1993,1994). Hence, the higher levels of Na⁺, Cl⁻, Mg²⁺, and Ca²⁺ in seminal plasma of *A. persicus* could be related to a higher secretory activity of the spermatic ducts of the Persian sturgeon, in comparison to that other sturgeon species. The Na⁺, K⁺ and Cl⁻ ions, like that in teleost fishes, is predominate in sturgeon seminal plasma (Linhart et al., 1991, Gallis et al., 1991; Billard et al., 1995a; Toth et al., 1997; Ciereszko et al., 2000). However, their concentrations in sturgeon fishes are lower than that in teleost seminal plasma (Table 3). The Mg²⁺ level is slightly different between sturgeon and teleost fishes (Table 3). The Ca²⁺ concentration in the seminal plasma of sturgeon fishes is lower than that in carps, but is close to that in cold water fish, especially to that in Salmonidae (Table 3). The differences between the results obtained dewing the present study and those obtained by other researchers could be related to several parameters including, spawning time of fish species (Emri et al., 1998), frequent contamination of semen
by urine and/or water during stripping (Perchec et al., 1995), phagositosis of sperm in the testis during degeneration stage of spermatogenesis (Alavi & Cosson, 2005), thinning and hydration of semen during spermiation period (Morisawa et al., 1979) and different environmental conditions during the spawning season (Emri et al., 1998). This study confirms again that Na⁺/K⁺ ratio in sturgeon seminal plasma is higher than that of salmonids and carps. The ratio is about 10 the same as in seminal plama of A. baeri (Gallis et al., 1991). This parameter, probably, explains the longer duration of sperm motility in A. persicus in comparison with that in Cyprinids and Salmonids. However, the reason for longer motility period in sturgeon sperm compared with that of freshwater teleosts is not clear, as the ratio decreases to average 4 in the Lake sturgeon, A. fulvecence (Toth et al., 1997).

Table 4: The ionic contents of the seminal plasma of some unidentified fishes, Cyprinids, Salmonids, and two sturgeon species, Lake sturgeon and Siberian sturgeon according to the literatures.

<table>
<thead>
<tr>
<th>Species</th>
<th>K⁺</th>
<th>Na⁺</th>
<th>Ca²⁺</th>
<th>Cl⁻</th>
<th>Mg²⁺</th>
<th>Author(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fishes</td>
<td>32-86</td>
<td>75-175</td>
<td>1-2</td>
<td>112-183</td>
<td>1-2</td>
<td>Ciereszko et al., 2000</td>
</tr>
<tr>
<td>Cyprinids</td>
<td>39-78</td>
<td>94-107</td>
<td>0.3-12.5</td>
<td>96-110.62*</td>
<td>0.02-1.2</td>
<td>Billard et al., 1995; Kruger et al., 1984</td>
</tr>
<tr>
<td>Salmonids</td>
<td>20-66</td>
<td>103-140</td>
<td>0.3-2.6</td>
<td>135*</td>
<td>0.8-3.6</td>
<td>Billard et al., 1995; Schlesk &amp; Kahmann, 1938</td>
</tr>
<tr>
<td>Acipenser baeri</td>
<td>2.5±0.3</td>
<td>28±0.7</td>
<td></td>
<td></td>
<td></td>
<td>Gallis et al., 1991</td>
</tr>
<tr>
<td>Acipenser fulvecence</td>
<td>5.78±0.49*</td>
<td>25.6±2.8*</td>
<td>0.16±0.05*</td>
<td>5.41±2.79*</td>
<td>0.21±0.02*</td>
<td>Toth et al., 1997; 1993; ***, 1994</td>
</tr>
</tbody>
</table>

The correlation between sperm density and motility:

Tvedt et al., (2001) observed no effect of sperm density on sperm motility in Atlantic halibut. This suggests that a combined sperm ejaculated from different males can be used in sturgeon artificial reproduction.
The correlation between seminal plasma indices and sperm motility:

Kruger et al. (1984) and Lahnsteiner et al., (1997) have reported significant positive correlations between Na⁺- Osmolality and Cl⁻ - Osmolality, in *Cyprinus carpio* and *Alburnus alburnus* seminal plasma, respectively. Probably, the Na⁺ and Cl⁻ are the main electrolytes having a major role in maintaining the osmolality of the seminal plasma (Morisawa et al., 1979) and the viability of the spermatozoa *in vivo* (Kruger et al., 1984), before it being released to the environmental medium and activation during the spawning. However, our knowledge on the changes of the ionic content seminal plasma as well as intracellular ionic content could be used to improve the cryopreservation techniques in fish sperm. Although, correlation coefficients between Na⁺ and K⁺ levels in the seminal plasma with percentage of motile spermatozoa are close to those found by Lahnsteiner et al., (1997) in *Alburnus alburnus*: (R=0.735, 0.471 and 0.572, respectively), but it is uncertain why there were no relationship between ionic composition of seminal plasma with sperm motility. However, like in other sturgeon (Toth et al., 1997 ; Billard, 2000) and teleost species (Scott & Baynes, 1980 ; Kruger et al., 1984 ; Lahnsteiner et al., 1997), the percentage of motile cells and total duration of sperm motility of *A. persicus* spermatozoa increases by decreasing the level of K⁺ ion and increasing of Na⁺ ion level in the seminal plasma and the osmolality of the seminal plasma. However, the biochemical interactions of ions in the seminal plasma, their influence on the spermatozoa membrane potential, mechanisms of inhibition of spermatozoa in the seminal plasma or sperm duct, and initiation of sperm after releasing to the surrounding medium at molecular and cellular levels are not clear.

In conclusion, the present study on the quality of *A. persicus* seminal plasma and its relationships with motility parameter determines that a seminal plasma containing Na⁺ (55-65 mM l⁻¹), K⁺ (very low level, <3mM), Ca²⁺ (approximately 0.8 mM l⁻¹) and Na⁺: K⁺ ratio of 11-13 and with the osmolality in the range of 80-90 mOsmol Kg⁻¹ seems to be the optimum quality of semen to be used in artificial fertilization of sturgeon species. But, a detailed study on the effect of pH of seminal plasma on motility of sperm is necessary to improve the fertilization procedure.
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