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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 (Ca^{2+}) and Magnesium (Mg^{2+}) 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 $8.34\pm1.38\times10^{9}$ spz/ml. The Sodium/Potassium and Calcium/Potassium ratios were 9.02 and 0.13,

•**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^- ($r^2= 0.584$, $P<0.05$). 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 ($r^2= 0.015$, $P>0.05$). 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 consider in creating an optimal medium for artificial fertilization and for cryopreservation (Suquet *et al.*, 2000 ; Billard *et al.*, 1995_b ; 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.*, 1995_a) and in sturgeon (Gallis *et al.*, 1991; Billard, 2000; Alavi, 2003; Alavi *et al.*, $2002_{a,b}$, 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.*, 1995_a). The change in seminal plasma osmolality is in correlation with thinning (hydration) of sperm during spermiation (Morisawa *et al.*, 1979).

The correlation between the composition of the seminal plasma and the potential for motility of spermatozoa has been investigated in a few species, only; Hwang & Idler, (1969), Billard & Cosson, (1992), Lahnsteiner *et al.*, (1996, 1997, 1998) in teleosts and Gallis *et al.*, (1991), Cosson & Linhart (1996), Toth *et al.*, (1997), Ingermann *et al.*, (2002), Linhart *et al.*, 2003), Alavi (2003) in sturgeons.

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 O_2 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 freezed 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 Technican (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 $8.34\pm1.38\times10^{9}$spz/ml. The osmolality of seminal plasma ranged from 42.00 to 111.00, averaging 82.56±8.10mOsmol 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±6.82mM) and the concentration of Cl^{-} (21.11±5.41mM) was higher than K^{+} that of ions (6.92±0.88mM) (Table 1). The concentrations of Ca^{2+} and Mg^{2+} were 0.79±0.03 and 0.52±0.03mM, 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 ($\times 10^9$ spz/ml) in the Persian sturgeon, *A. persicus*

Parameters	Min	Max	Mean	SEM
Sperm density	4.29	16.06	8.34	1.38
Osmolality	42.00	111.00	82.56	8.10
Na^+	21.00	80.00	62.44	6.82
K^+	3.70	11.80	6.92	0.88
Cl^-	2.00	43.00	21.11	5.47
Mg^{2+}	0.41	0.62	0.52	0.03
Ca^{2+}	0.66	0.91	0.79	0.03

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

	K^+	Cl^-	Mg^{2+}	Ca^{2+}	Osmolality
Na^+ mM l^{-1}	0.2587	0.5844b	0.0186	0.0001	0.9053a
K^+ mM l^{-1}	--------	0.2959	0.0140	0.0718	--------
Mg^{2+}mM l^{-1}	--------	0.0992	--------	--------	--------
Ca^{2+} mM l^{-1}	--------	0.0177	0.2099	--------	--------
Osmolality (mOsmol Kg^{-1})	0.2022	0.4922b	0.0081	0.0171	--------

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).

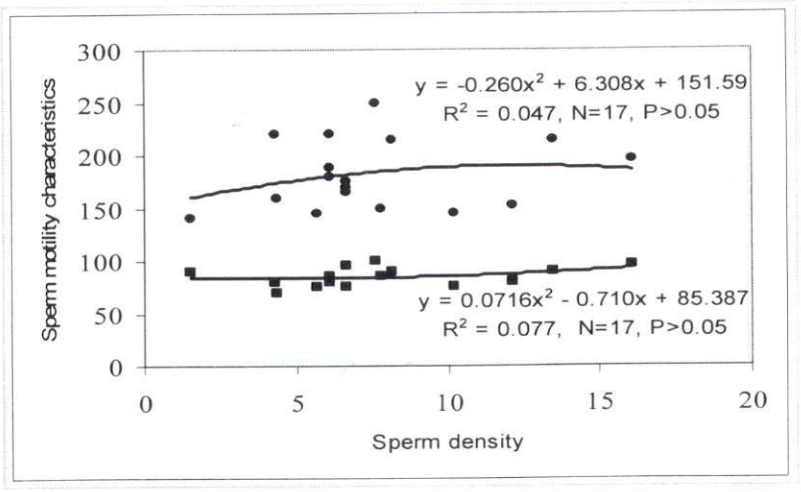

Figure 1: The relationship between sperm density ($\times 10^9$ spz/ml) and sperm motility characteristics (the percentage of motile cells [%, ■] and total period of motility [Sec, •]) of *Acipenser persicus* (N=17 samples from 6 males, ANOVA, P>0.05).

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 spermazozoa 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 70mM 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 Ca^{2+} 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 Mg^{2+} concentration was about 0.5mM L^{-1} (Figure 2f). Also, the percentage of motile cells decreased, when the Mg^{2+} 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 Ca^{2+}/ K^+ ratio in the seminal plasma (Figure 2h). By increasing the Ca^{2+}/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 Ca^{2+}/K^+ (panel h)] and sperm motility characteristics (the percentage of motile cells [%, ■] and total period of motility [Sec, •] in *Acipenser persicus*.

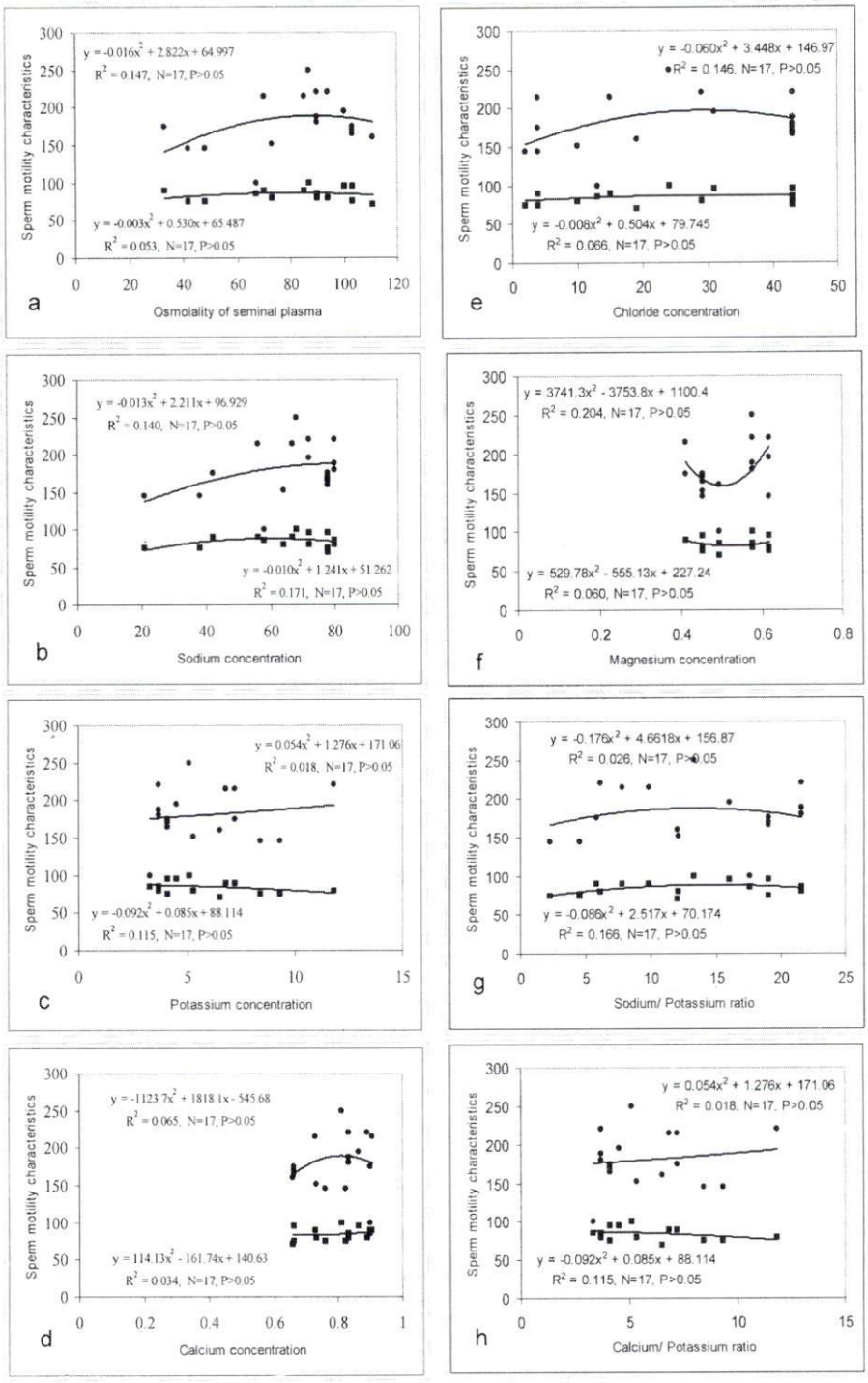
Sperm motility characteristics
y = -0.016x² + 2.822x + 64.997
R² = 0.147, N=17, P>0.05
y = -0.003x² + 0.530x + 65.487
R² = 0.053, N=17, P>0.05
Osmolality of seminal plasma
a
y = -0.060x² + 3.448x + 146.97
R² = 0.146, N=17, P>0.05
y = -0.008x² + 0.504x + 79.745
R² = 0.066, N=17, P>0.05
Chloride concentration
e
y = -0.013x² + 2.211x + 96.929
R² = 0.140, N=17, P>0.05
y = -0.010x² + 1.241x + 51.262
R² = 0.171, N=17, P>0.05
Sodium concentration
b
y = 3741.3x² - 3753.8x + 1100.4
R² = 0.204, N=17, P>0.05
y = 529.78x² - 555.13x + 227.24
R² = 0.060, N=17, P>0.05
Magnesium concentration
f
y = 0.054x² + 1.276x + 171.06
R² = 0.018, N=17, P>0.05
y = -0.092x² + 0.085x + 88.114
R² = 0.115, N=17, P>0.05
Potassium concentration
c
y = -0.176x² + 4.6618x + 156.87
R² = 0.026, N=17, P>0.05
y = -0.086x² + 2.517x + 70.174
R² = 0.166, N=17, P>0.05
Sodium/ Potassium ratio
g
y = -1123.7x² + 1818.1x - 545.68
R² = 0.065, N=17, P>0.05
y = 114.13x² - 161.74x + 140.63
R² = 0.034, N=17, P>0.05
Calcium concentration
d
y = 0.054x² + 1.276x + 171.06
R² = 0.018, N=17, P>0.05
y = -0.092x² + 0.085x + 88.114
R² = 0.115, N=17, P>0.05
Calcium/ Potassium ratio
h

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. 1.5×10^9spz/ml), Ahmadian (2000) (Mean 0.64×10^9spz/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^-, Mg^{2+}, and Ca^{2+} of the seminal plasma of *A. persicus* are higher than those of other Acipenserids species (*e.g.*, *A. baeri*, Gallis *et al.*, 1991; *A. fulvecsence*, 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. fulvecsence* (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^-, Mg^{2+}, and Ca^{2+} 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.*, 1995_a; 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 Mg^{2+} level is slightly different between sturgeon and teleost fishes (Table 3). The Ca^{2+} 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 semial plama of *A. baeri* (Gallis *et al.*, 1991). This parameter, probably, explains the longer duration of sperm motility in *A. persicus* in comparison whit 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. *fulvecsence* (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.

Species	K^+	Na^+	Ca^{2+}	Cl^-	Mg^{2+}	Author(s)
Fishes	32-86	75-175	1-2	112-183	1-2	Ciereszko *et al.*, 2000
Cyprinids	39-78	94-107	0.3-12.5	96-110.62*	0.02-1.2	Billard *et al.*, 1995_a *Kruger *et al.*, 1984
Salmonids	20-66	103-140	0.3-2.6	135*	0.8-3.6	Billard *et al.*, 1995_a *Schlenk & Kahmann, 1938
Acipenser baeri	2.5±0.3	28±0.7				Gallis *et al.*, 1991
Acipenser fulvecsence	5.78±0.49* 6.97±1.42**	25.6±2.8* 31.8±7.0**	016±0.05* 0.13±0.02**	5.41±2.79* 2.31±1.28**	0.21±0.02* 0.22±0.02**	Toth *et al.*, 1997*, 1993 ; **, 1994

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 containg Na^+ (55-65 mM l^{-1}), K^+ (very low level, <3mM), Ca^{2+} (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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References

Ahmadian, N. , 2000. Comparative study on fertilization of egg in the Persian sturgeon, *Acipenser persicus*, using sperm activation solution. M.Sc Thesis, Tarbiat Modarres University. 55P.

Alavi, S.M.H. , 2003. Comparative study on motility and fertilizing ability of the Persian sturgeon, *Acipenser persicus*, spermatozoa between freshwater and saline solutions. M.Sc thesis, Dept. of Fisheries and Environmental Sciences, University of Tehran. 105P.

Alavi, S.M.H. and Cosson, J. , 2002. Sperm motility in fishes: (III) Mechanisms of activation of the motility of spermatozoa. 26th Annual Larval Fish Conference. 22-26 July, Bergen, Norway, Page 29.

Alavi, S.M.H. and Cosson, J. , 2005. Sperm motility in fishes: (II) Effects of ions and osmotic pressure. Cell Biology International, In press.

Alavi, S.M.H. ; Cosson, J. ; Karami, M. ; Mojazi Amiri, B. and Akhoundzadeh M.A. , 2004. Spermatozoa motility in the Persian sturgeon, *Acipenser persicus*: Effects of pH, dilution rate, ions and osmolality. Reproduction, **128:** 819-828

Alavi, S.M.H. ; Mojazi Amiri, B. ; Cosson, J. ; Pourkazemi, M. and Karami, M. , 2002a. A preliminary investigation on motility of *Acipenser persicus*

spermatozoa: A comparative study between freshwater and saline solutions at different dilution rate. The 2nd National-Regional Symposium on Sturgeon, October 26-28, Rasht, Iran, pp.128-130.

Alavi, S.M.H. ; Mojazi Amiri, B. and Pourkazemi, M. , 2002b. Total period of motility of Acipenser persicus spermatozoa in freshwater and saline solution. Iran. J. Fish. Sci., 4**(1):**68-76.

Billard, R. , 2000. Biology and control of reproduction of sturgeon in fish farm. Iran. J. Fish. Sci., **2:**1-20

Billard, R. and Cosson, M.P. , 1992. Some problems related to the assessment of sperm motility in freshwater fish. J. Exp. Zoology, **261:**122-131

Billard, R. ; Cosson, J. ; Crim, L.W. and Suquet, M. , 1995a. Sperm physiology and quality. *In*: Brood stock management and egg and larval quality. N.R. Bromage and R. J. Roberts, (Eds). Blackwell Science. pp.25-52.

Billard, R. ; Cosson, J. ; Perchec, G. and Linhart, O. , 1995b. Biology of sperm and artificial reproduction in carp. Aquaculture, **124:**95-112.

Billard, R. ; Cosson, J. ; Fierville, F. ; Brun, R. ; Rouault, T. and Williot, P. , 1999. Motility analysis and energetics of the Siberian sturgeon, *Acipenser baerii*, spermatozoa. J. Applied Ichthyol. **15:**199-203.

Ciereszko, A. and Dabrowski, K. , 1993. Estimation of sperm concentration of rainbow trout, whitefish and yellow perch using a sperctrophotometric technique. Aquaculture. **109:**367-373.

Ciereszko, A. ; Glogowski, J. and Dabrowski, K. , 2000. Biochemical characteristics of seminal plasma and spermatozoa of freshwater fishes. *In*: Cryopreservation of Aquatic Species. Tiersch, T.R. and P.M. Mazik, (Eds). World Aquaculture Society, Baton Rouge, Louisiana. pp.20-48.

Cosson, J. ; Linhart, O. , 1996. Paddlefish (*Polyodon spathula*) spermatozoa: Effects of potassium and pH on motility. Folia Zoologica, **45:**361-370.

Cosson, J. ; Billard, R. ; Gibert, C. ; Dreanno, C. and Suquet, M. , 1999. Ionic factors regulating the motility of fish sperm. In The male gamete: From basic to clinical applications, C. Gagnon, (Ed). Cache Rive Press. pp.61-186.

Emri, M. ; Marian, T. ; Tron, L. ; Balkay, L. and Krasznai, Z. , 1998. Temperature adaptation changes in spermatozoa and seminal plasma of common carp, *Cyprinus carpio*, without affecting sperm motility. Aquaculture. **167:**85-94.

Gallis, J.L. ; Fedrigo, E. ; Jatteau, P. ; Bonpunt, E. and Billard, R. , 1991. Siberian sturgeon spermatozoa: Effects of dilution, pH, osmotic pressure, sodium and potassium ions on motility. *In*: P. Williot (Ed), Acipenser, Cemagref, Bordeaux, pp.143-151.

Ginsburg, A.S. , 1968. Fertilization of fishes and the problem of polyspermy. Moscow. Academy of Science USSR; Translation: NOOAA and National Science Fondatio. New York. 354P.

Hwang, P.C. and Idler, D.R. , 1969. A study of major cations, osmotic pressure, and pH in seminal components of Atlantic salmon. J. Fish. Res. Board Can., **26:**413-419.

Ingermann, R. ; Holcomb, M. ; Robinson, M.L. and Cloud, J.G. , 2002. Carbon dioxide and pH affect sperm motility of white sturgeon (*Acipenser transmontanu0s*). J. Exp. Biol., **205:**2885-2890.

Kohneshahri, M. and Azari Takami, G. , 1974. Artificial Propagation of Sturgeons. Tehran University Publication, Tehran.

Kruger, J.C. ; Smith, G.L. ; Van Vuren, J.H.J. and Ferreira, J.T. , 1984. Some chemical and physical characterestics of the semen of *Cyprinus carpio* and *Oreochromis mossambicus*. J. Fish Biol., **24:**263-272.

Lahnsteiner, F. ; Patzner, R.A. and Weismann, T. , 1993. The spermatic ducts of salmonid fishes (*Salmonidae, Teleostei*). Morphology, histochemistry and composition of the secretion. J. Fish Biol., **42:**79-93.

Lahnsteiner, F. ; Patzner, R.A. and Weismann, T. , 1994. The testicular main duct and the spermatic duct in some cyprinid fishes. II. Composition of seminal fluid. J. Fish Biol., **44:**459-467.

Lahnsteiner, F. ; Berger, B. ; Weismann, T. and Patzner, R.A. , 1996. Motility of spermatozoa of *Alburnus alburnus* (*Cyprinidae*) and its relationship to

seminal plasma composition and sperm metabolism. Fish Physiology and Biochemistry. **15:**167-179.

Lahnsteiner, F. ; Berger, B. ; Weismann, T. and Patzner, R.A. , 1997. Sperm motility and seminal composition in the Turbot, *Lota lota*. J. Appl. Ichthyol. **13:**113-119.

Lahnsteiner, F. ; Berger, B. ; Weismann, T. and Patzner, R.A. , 1998. Determination of semen quality of the rainbow trout by sperm motility, seminal plasma parameters and spermatozoal metabolism. Aquaculture. **163:** 163-181.

Linhart, O. ; Cosson, J. ; Mims, S.D. ; Rodina, M. ; Gela, D. and Shelton, W.L. , 2003. Effects of ions on the motility of fresh and demembranate spermatozoa of common carp (*Cyprinus carpio*) and paddlefish (*Polyodon spathula*). Fish Physiology and Biochemistry. **28:**203-205.

Linhart, O. ; Slechta, V. and Slavik, T. , 1991. Fish sperm composition and biochemistry. Bulletin of the institute of zoology. Academia Sinica. Monograph. **16:**285-311

Marshall, W.S. ; Bryson, S.E. and Idler, R.D. , 1989. Gonadotropin stimulation of K^+ secretion and Na^+ absorption by brook trout, *Salvelinus fontinalis*, sperm duct epithelium. Gen. Comp. Endocrinol. **75:**118-128.

Morisawa, M. ; Hirano, T. and Suzuki, K. , 1979. Changes in blood and seminal plasma composition of the mature salmon (*Oncorhynchus keta*) during adaptation to freshwater. Comp. Biochem. Physiol. **64:**325-329.

Perchec, G. ; Jeulin, C. ; Cosson, J. ; Andre, F. and Billard, R. , 1995. Relationship between sperm ATP content and motility of carp spermatozoa. J. Cell Science. **108:**747-753.

Pohl-Branscheid, M. and Holtz, W. , 1990. Control of spawning activity in male and female rainbow trout (*Oncorhynchus mykiss*) by repeated foreshortened seasonal light cycles. Aquaculture. **86:**93-104.

Schlenk, W. and Kahmmann, H. , 1938. The chemical composition of seminal fluids and their physiological importance study with trout sperm. Biochemical Zool. **295:**283-301.

Scott, A.P. and Baynes, S.M. , 1980. A review of the biology, handling and storage of slmonid spermatozoa. J. Fish Biol. **17:**707-739.

Suquet, M. ; Omnes, M.H. ; Normant, Y. and Fauvel, D.K. , 1992. Assessment of sperm concentration and motility in Turbot, *Scophthalmus maximus*. Aquaculture. **101:**177-185.

Suquet, M. ; Billard, R. ; Cosson, J. ; Dorange, G. ; Chauvaud, L. ; Mugnier C. and Fauvel, C. , 1994. Sperm features in turbot (*Scophthalmus maximus*): a comparison with other freshwater and marine fish species. Aquat. Living Resour. **7:**283-294.

Suquet, M. ; Dreanno, C. ; Dorange, G. ; Normant, Y. ; Quemener, L. ; Gaignon, J. L. and Billard, R. , 1998. The aging phenomenon of Turbot, *Scophthalmus maximus*, spermatozoa: Effects on morphology, motility and concentration, intracellular ATP content, fertilization and storage capacities. J. Fish Biol. **32:**31-41.

Suquet, M. ; Dreanno, C. ; Fauvel C. ; Cosson, J. and Billard R. , 2000. Cryopreservation of sperm in marine fish. Aquaculture Research. **31:**231-243.

Toth, G.P. ; Ciereszko, A. ; Christ, S.A. and Dabrowski, K. , 1997. Objective analysis of sperm motility in the Lake sturgeon, *Acipenser fulvescens*: Activation and inhibition conditions. Aquaculture. **154:**337-348.

Tsvetkova, L.I. ; Cosson, J. ; Linhart, O. and Billard, R. , 1996. Motility and fertilizing capacity of fresh and frozen-thawed spermatozoa in sturgeon, *Acipenser baeri* and *A. ruthenus*. J. Appl. Ichthyol. **12:**107-112.

Tvedt, H.B. ; Benefy, T.J. ; Martin-Robichaud, D.J. and Poer, J. , 2001. The relationship between sperm density, spermatocrit, sperm motility and fertilization success in Atlantic halibut, *Hippoglossus hippoglossus*. Aquaculture. **194:**191-200.