

Physiological Correlation between Sperm Motility and Seminal Plasma Composition in the Persian sturgeon, *Acipenser persicus*

Mohammad Sadegh Aramli^{1*}, Mohammad Reza Kalbassi¹, Rajabmohammad Nazari²

1. Aquaculture Department, Tarbiat Modares University, Noor, Mazandaran, Iran

2. Rajaei Sturgeon Hatchery Center, Sari, P.O. Box 833, Sari, Mazandaran, Iran

Abstract

Knowledge of the reproductive physiology of wild sturgeon populations is critical to ensure the survival of this unique group of animals. This experiment was designed to explore the physiological relationships between the seminal plasma composition (ionic and organic contents as well as osmolality) and sperm motility in the Persian sturgeon (*Acipenser persicus*). In this regard, 17 brood fish were injected intramuscularly by LHRH-A2 ($5\mu\text{gKg}^{-1}$) for spermiation induction. According our results, the seminal plasma contained 59.53 ± 2.56 mM/l sodium, 9.1 ± 1.42 mM/l chloride, 4.72 ± 0.3 mM/l potassium, 1.45 ± 0.075 mM/l calcium and 0.7 ± 0.072 mM/l magnesium. 0.11 ± 0.02 g/dl total protein, 22.18 ± 4.16 mg/dl glucose, 6.67 ± 1.04 mg/dl cholesterol and 15.2 ± 0.65 mg/dl triglyceride. The total duration of sperm movement was obtained 288.01 ± 14.44 second. The osmolality of seminal plasma ranged from 47 to 176 mOsmol kg^{-1} . Significant positive relationships were determined between sperm motility vs. K^+ ion ($r=0.718$, $p<0.01$) and total protein ($r=0.670$, $p<0.01$). No significant correlations were found between sperm motility and others composition of the seminal plasma. Presented data could be considered as a complementary study for developing special extenders and protectant solutions for improving artificial fertilization in this valuable species.

Key words: Spermatozoa motility, seminal plasma composition, *Acipenser persicus*.

1- Introduction

Sturgeons represent one of the most threatened fish in the world, with 18 of the 27 recognized species of *Acipenseriformes* listed as endangered or critically endangered (IUCN 2010). Because of their life-history characteristics including slow growth speed and high of sexual maturity time, sturgeons are particularly sensitive to low levels of exploitation and habitat destruction (41, 9). Reliable information on the reproductive physiology and biology of wild populations of sturgeon is therefore critical to ensure the perpetuation of this ancient group of animals (21).

Persian sturgeon, *Acipenser persicus* (Borodin 1897) is an anadromous fish which widely distributed

in the southern Part of the Caspian Sea (13). Similar to most sturgeons, this species has become an endangered species because of overfishing for meat and caviar production, habitat degradation and water pollution (32). For these reasons, most researches in recent years have been focused on the culture of Persian sturgeon for restocking and conservation of their natural populations (6, 7, 4, 2, 1).

In aquaculture industry, it is necessary to assessment sperm quality in order to determine the optimal time for sperm collection and improvement of storage producers of sperm and finally increase the efficiency of artificial propagation (5). Sperm motility is a key factor determining the quality and fertilizing ability of sperm which commonly expressed as percentage and duration of motile sperm after activation (11, 29, 43). Sperm in fish species remain immobile in seminal plasma and are activated when released into water or extender solutions (3). In this regarding, osmolality and seminal plasma component

*Corresponding Address: P.O. Box: 46414-356, Aquaculture Department, Faculty of Marine Sciences, Tarbiat Modares University, Noor, Mazandaran Province, Iran, Email: msaramli@gmail.com.

prevent sperm motility in fish sperm duct (10, 46). In addition, seminal plasma produced by the sperm duct provides a suitable microenvironment that maintains the viability of sperm after their release from the testes into the sperm duct and subsequently after discharge of sperm into the aquatic environment (17).

The study of correlation between seminal plasma composition and sperm motility has been reported in some species: Atlantic salmon, *Salmo salar* (22), Common carp, *Cyprinus carpio* (23), Bleak, *Alburnus alburnus* and Rainbow trout, *Oncorhynchus mykiss* (25, 26), Lake sturgeon, *Acipenser fulvescens* (47), Grass carp, *Ctenopharyngodon idella* (14), Chinook salmon, *Oncorhynchus tshawytscha* (42), and *Salmo trutta macrostigma* (15). Also, some papers have been already published in relation to Persian sturgeon (1, 2, 4), but no information is available regarding organic composition and their relationships in this species. In the completion of previous studies on Persian sturgeon semen, this study was conducted to determine some parameters of the seminal plasma including osmolality, ionic and organic composition in *A. persicus* and their physiological relationships with sperm motility.

2- Materials and methods

2-1- Brood fish and sampling

The experiments were carried out during March-April 2011. The fish were obtained from commercial fishing for restocking purposes and therefore no fish were solely used or sacrificed for this study. Male Persian sturgeon broodstocks were captured using gillnets (length 18 m, width 5.4 m, mesh size 15 cm) from Caspian Sea (water temperature 10-11.5 °C) and transported by boat (6 hr duration) to Sturgeon Hatchery Center (Sari, Mazandaran, Iran; Lat 36°37' N, Long 53°05' E) and kept for 5 days in 3.5 m deep 1500 m² holding ponds at 12.5–13 °C, with a freshwater supply. Water temperature, oxygen content and pH were at 17–18 °C, >5.1 mg l⁻¹ and 7.6-7.9, respectively during the experiments. Fish were injected with an analogue of releasing hormone LHRH-A₂ (D-Ala⁶ GnRH Pro⁹-NEt), (34) at a dose of 5 µg kg⁻¹ of body weight (BW). Spermiation occurred within 18 h after hormonal stimulation and the semen was collected using a syringe with attached rigid tubing inserted into the urogenital opening (to avoid contamination by urine). Semen was stored in glass tubes on ice (+4°C) during transportation to a laboratory (up to 3 h).

2-2- Evaluation of sperm motility

For the evaluation of motility, 10 µL of semen was placed on a microscope slide and 100 µL of distilled water was added. Total period of motility was estimated using a sensitive chronometer and under a light microscopy until approximately 90% of the sperm stopped their progressive movement (14).

2-3- Determination of seminal plasma composition

For this purpose, the semen samples were centrifuged (Spectrafuge16M, USA) using a Two-step method, first at 500 rpm for 2 min, and second at 3000 rpm for 10 min. The supernatants were collected and frozen in -20 °C until use for analysis. The concentration of magnesium (Mg²⁺), calcium (Ca²⁺) and chloride (Cl⁻) were measured with colorimetric procedure using an Auto analyzer (BT 3500 plus, Italy). Potassium (K⁺) and sodium (Na⁺) were determined by flame photometer (Jenway, England). The osmolality of seminal plasma was measured in duplicates per sample with osmometer (Osmomat 030-m, Germany) using a freezing point depression. Metabolites (glucose, triglyceride and cholesterol and total protein) were determined by auto analyzer (Eurolyser vet, Austria) using commercial clinical investigation kits (Pars Azmoon Kit, Tehran, Iran). All assays were carried out according to the protocol established by the manufacturer.

2-4- Statistical analysis

To carry out data analysis we made use of SPSS 11.5 software (Chicago, IL, USA). Results of sperm quality parameters from individual fish were analyzed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test (DMRT) at P<0.05. Correlations between sperm motility and seminal plasma composition were estimated using Pearson's correlation test.

3- Results

Descriptive statistics of sampled sperm in Persian sturgeon are shown in Table 1. The total duration of sperm motility was estimated to be 288.01±14.44 second. The seminal plasma osmolality was 86.90±4.22 mOsmkg⁻¹. The ionic content of the seminal plasma was found to be rather variable and Na⁺, Cl⁻ and K⁺ were ions predominate in Persian sturgeon. The mean of glucose, triglyceride and cholesterol were obtained 22.18±4.16, 15.2±0.65 and 6.67±1.04 mg dL⁻¹ respectively. Total protein content varied from 0.1 to 0.6g dL⁻¹. The linear relationships between sperm motility and seminal plasma

Composition are shown in Table 2. The K^+ ion correlated positively with sperm motility ($r=0.718$, $P<0.01$) (Figure 1c). A significant positive correlation was also found between the sperm motility and the total protein content of the seminal plasma ($r=0.670$, $P<0.01$) (Figure 2a). No significant correlation was found between sperm motility and other parameters of seminal plasma. Significant

correlations were obtained between some seminal plasma indices: K^+ and Ca^{2+} ($r=0.492$, $P<0.05$), K^+ and Na^+ ($r=0.563$, $P<0.05$); Cl^- and Na^+ ($r=0.770$, $P<0.01$); osmolality and Na^+ ($r=0.544$, $P<0.05$), osmolality and Cl^- ($r=0.696$, $P<0.01$); triglyceride and Na^+ ($r=0.506$, $P<0.05$), triglyceride and Cl^- ($r=0.676$, $P<0.01$); cholesterol and Ca^{2+} ($r=0.655$, $P<0.01$).

Table 1 Descriptive statistics of sampled fish, sperm motility and seminal plasma characteristics in Persian sturgeon. (n=17)

Parameters	Minimum	Maximum	Mean	SEM
Biometry of sampled fish				
Fish length (cm)	105.00	175.00	143.38	5.47
Fish weight (Kg)	12.00	21.00	15.81	0.57
Total duration of sperm (second)	76.33	456.66	288.01	14.44
Sodium (mEq L ⁻¹)	72.40	102.00	59.53	2.56
Chloride (mEq L ⁻¹)	0.30	35.00	9.10	1.42
Potassium (mEq L ⁻¹)	1.90	10.5.0	4.72	0.31
Calcium (mg dL ⁻¹)	0.80	4.00	1.45	0.075
Magnesium (mEq L ⁻¹)	0.10	1.90	0.70	0.07
Osmolality (mOsm Kg ⁻¹)	47.00	176.00	86.90	4.22
Glucose (mg dL ⁻¹)	0.30	111.60	22.18	4.16
Cholesterol (mg dL ⁻¹)	0.00	38.90	6.67	1.04
Triglyceride (mg dL ⁻¹)	7.30	32.70	15.20	0.65
Total protein (g dL ⁻¹)	0.10	0.60	0.11	0.02

Table 2 Linear relationships (r) (Pearson's correlation test) between spermatozoa motility and seminal plasma characteristics of Persian sturgeon sperm.

	Na ⁺	Cl ⁻	K ⁺	Ca ²⁺	Mg ²⁺	Sperm motility	Osmolality	Protein	Glucose	Triglyceride
Cl ⁻	0.770**									
K ⁺	0.563*	0.256								
Ca ²⁺	0.125	-0.064	0.492*							
Mg ²⁺	0.250	-0.258	0.391	-0.349						
Sperm motility	0.257	-0.07	0.718**	0.180	0.106					
Osmolality	0.544*	0.696*	0.014	-0.176	-0.371	-0.030				
Protein	-0.253	-0.203	-0.031	-0.120	0.388	0.670**	-0.413			
Glucose	-0.080	0.321	-0.119	-0.309	0.004	-0.044	0.429	-0.011		
Triglyceride	0.506*	0.676**	0.262	-0.242	-0.361	0.059	0.522	-0.342	0.142	
Cholesterol	-0.205	-0.296	0.066	0.665**	0.015	0.092	-0.393	0.111	-0.211	-0.324

*Significant at $P<0.05$

** Significant at $P<0.01$

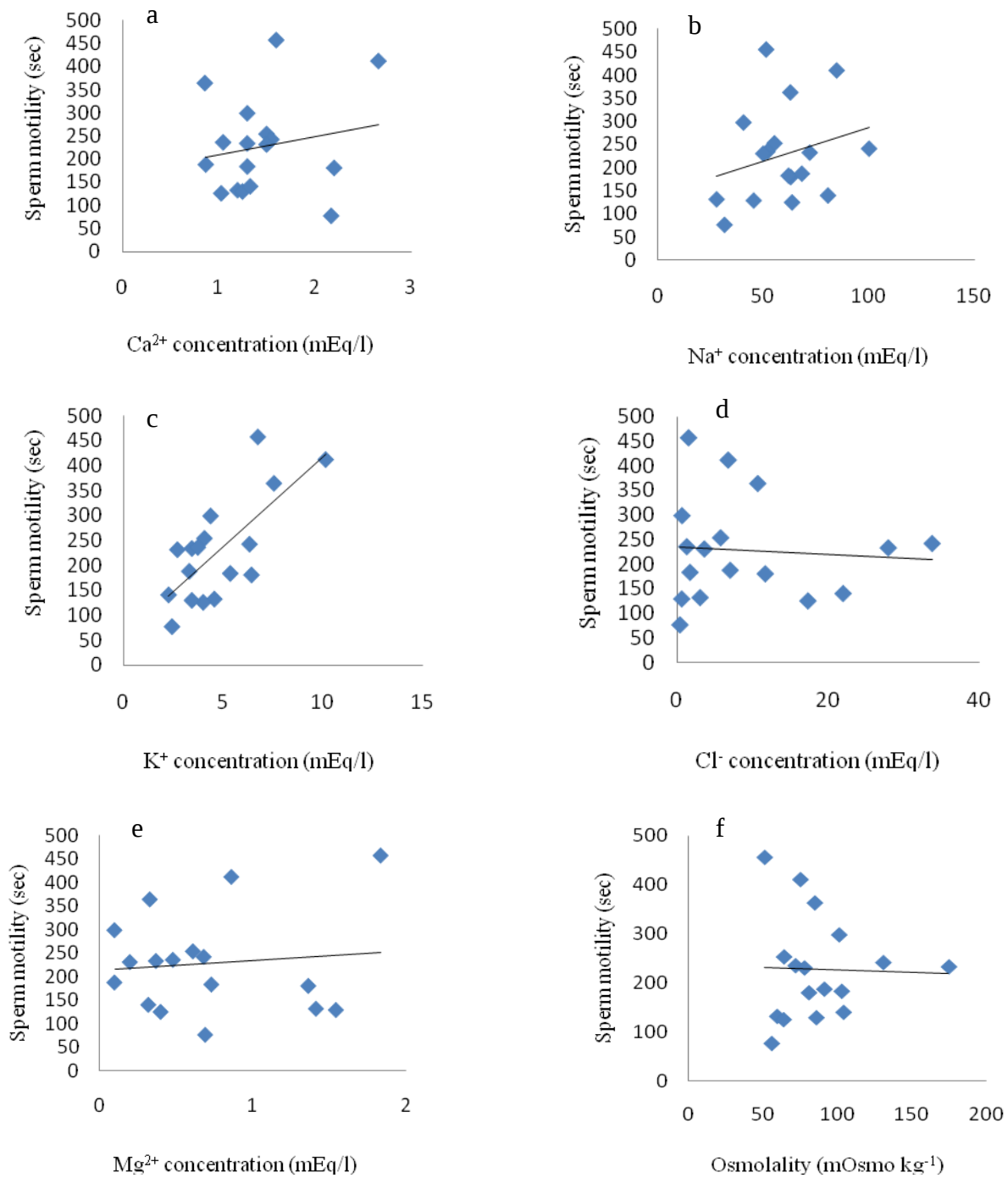


Figure 1 Relationships between spermatozoa motility with ionic composition: (a) Ca^{2+} ($y=38.92x+171.5$, $r^2=0.03$), (b) Na^{+} ($y=1.446x+141.9$, $r^2=0.07$), (c) K^{+} ($y=35.98x+56.87$, $r^2=0.52$), (d) Cl^{-} ($y=0.714x+234.5$, $r^2=0.004$), (e) Mg^{2+} ($y=21.06x+213.1$, $r^2=0.01$) and (f) osmolality ($y=-0.103x+237.0$, $r^2=0.00$) of the seminal plasma in Persian sturgeon (n= 17, ANOVA).

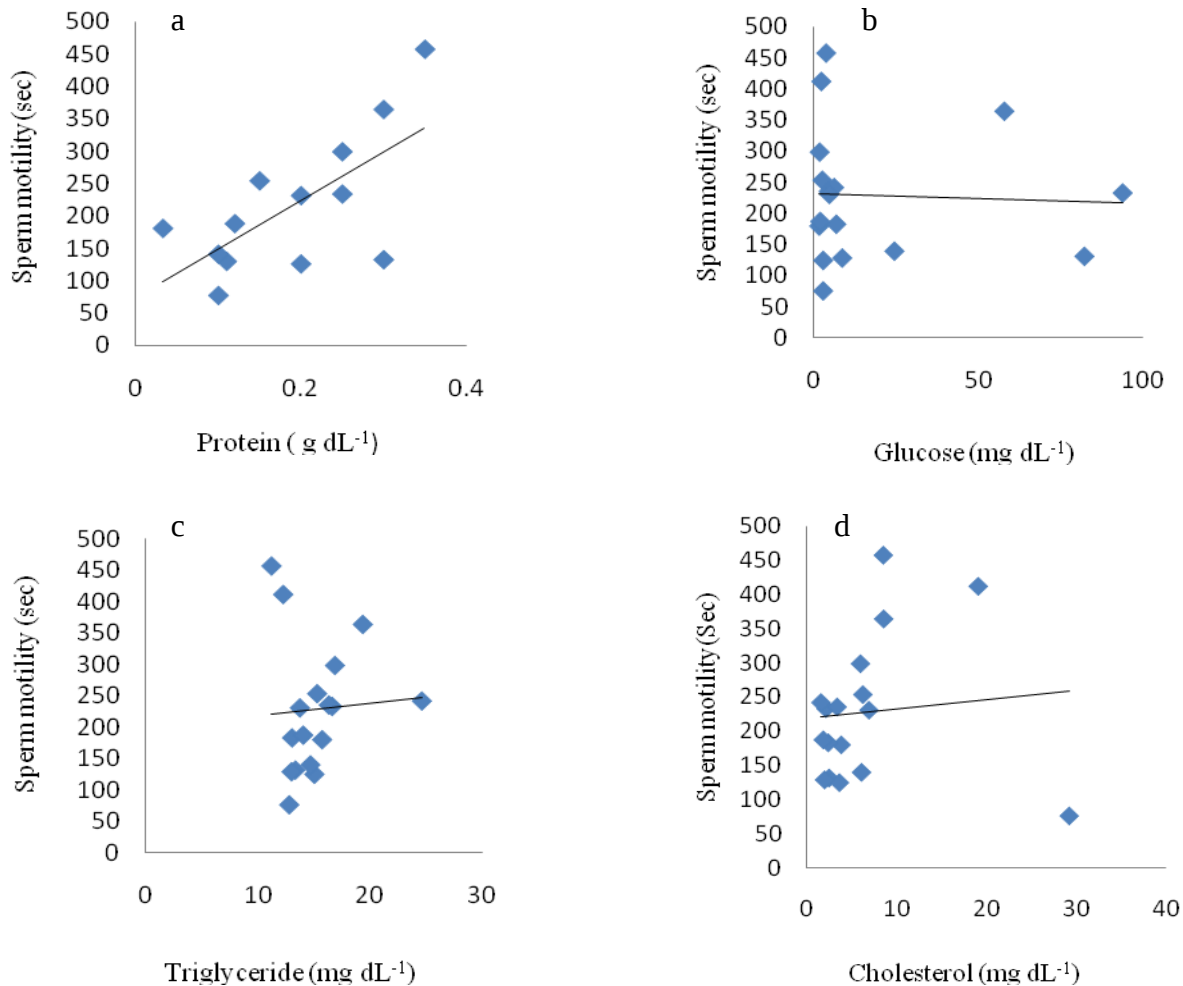


Figure 2 Relationships between spermatozoa motility and organic composition: (a) protein ($y=749.9x+73.68$, $r^2=0.45$), (b) glucose ($y=-0.156x+230.8$, $r^2=0.001$), (c) triglyceride ($y=1.345x+219.0$, $r^2=0.01$), and (d) cholesterol ($y=1.947x+198.4$, $r^2=0.003$) of the seminal plasma in Persian sturgeon ($n=17$, ANOVA).

Discussion

In sturgeon fish the duration of sperm motility is higher in comparison with teleost fish. Alavi and Cosson (2006) (3) postulated, the clear differences such as Na^+/K^+ ratio and structural features of sperm, (volume and number of mitochondria is larger in sturgeons) could explain why sperm remain motile for longer period in sturgeons than in the other species. In this study, Na^+/K^+ ratio (12.61) was higher than the previously obtained value (9.02) by Alavi et al. (2004) (1). In comparison to other sturgeon, it was higher than Stellate sturgeon, *Acipenser stellatus* (3.70) and Lake sturgeon, *Acipenser fulvescens* (4.43) (31, 47) and lower than Russian sturgeon, *Acipenser gueldenstaedtii* (15.10) reported by Li et al. (2011)

(31) and starlet, *Acipenser ruthenus* (15.96), reported by Psenicka et al. (2008) (39). Most studies on several sturgeon species have shown that the duration of sperm movement is highly variable (12). Similar to others sturgeons, the spermatozoa motility of the Persian sturgeon is characterized by motility periods lasting longer than in teleost fishes. In present study, the mean of sperm motility (2 to 7 min) was lower than that in the starlet - *Acipenser ruthenus* (5 to 20 min) (48), but it was close to that reported by Drabkina (1961) (18) in the Russian sturgeon, *A. gueldenstaedtii* (3 to 6 min). The difference may be due to differences in several parameters such as feeding condition, age, environmental factors, time of spawning, composition of the seminal plasma (ions

concentration and osmolality) and dilution ratio (14, 3).

Seminal plasma has a unique composition that plays an important role in viability of sperm. The ionic and organic investigations of seminal plasma have generally been aid in evaluation of the reproductive ability in broodstocks (23). Our study detected that similar to other fish species, Na^+ , K^+ and Cl^- are predominant ions in the seminal plasma of Persian sturgeon but theirs concentrations are lower than the concentrations reported in teleost fishes (36, 23, 19, 28, 47, 16). On the other hand, the mean concentrations of sodium, calcium, magnesium, chloride in Persian sturgeon were higher in comparison with other sturgeons (31, 38). Probably, the high monvalent and divalent cations levels could be related to high- secretory activity in the Persian sturgeon spermatid duct. The seminal plasma osmolality in sturgeons is much lower than that of the teleost fish (3). In the present study, osmolality of the seminal plasma of *A. persicus* was higher than those reported for *A. ruthenus* (38), *A. stellatus*, and *A. gueldenstaedtii* (31). Similar to the ionic content, the osmolality can vary between individuals, which the variations in osmolality might be due to hydration of sperm (33), and hormonal stimulation of spermiation in the time of artificial propagation (40). In addition, ionic concentrations as well as osmolality differs among results reported by researchers could be related to environmental condition, frequency of stripping, and hormonal stimulation of spermiation (11, 17), and also result from contamination of sperm by urine (35).

Analysis of organic composition of seminal plasma is an important criterion for evaluation of sperm quality (11). The studies focused on organic indices of sperm in sturgeons are scare or limited. In this study, we assessed the metabolites characteristics of seminal plasma for generating an information database for improvement of storage methods in the Persian sturgeon sperm. Seminal plasma of Persian sturgeon similar to other sturgeons is characterized by much lower protein concentration (38). This study again confirmed that protein concentrations in the seminal plasma of most fish are much lower than in the other vertebrates (17). Low protein concentration indicates that low protein amounts are necessary for sperm in this species. The importance of glucose in fish sperm is not clear. The mean of glucose level in our study was $22.18 \pm 4.16 \text{ mg dL}^{-1}$. Soengas et al. (1993) (45) explained that the presence of glucose in seminal plasma has been related to the high energy demand of the testes during spermatogenesis. Triglycerides were provided energy requirements of

sperm during immotile storage and during the renewal phase after motility (24). Published results indicate that various lipid classes have been identified in seminal plasma and their contents vary among fish species (36, 37). The triglyceride level of seminal plasma has been reported in some species, such has $15.2 \pm 0.65 \text{ mg dL}^{-1}$ for Persian sturgeon (present study), $14.58 \pm 1.50 \text{ mg dL}^{-1}$ for Grass carp (14) and $8.0 \pm 2.84 \text{ mg dL}^{-1}$ for Rainbow trout, (44). In the present study, cholesterol has been detected in the seminal plasma of Persian sturgeon and its level was found to be $6.67 \pm 1.04 \text{ mg dL}^{-1}$. The role of cholesterol in sperm functioning has been investigated in numerous studies with the aim to improve the sperm deep-freezing technique and composition of sperm extenders (8). Generally, sperm structure and fertilizing ability depend on lipid composition in sperm cell membrane and seminal plasma. Lipids and cholesterol probably might have a protective function versus environmental changes when sperm is released to water or extender solutions.

Investigation and understanding of relationships between sperm motility and seminal plasma characteristics could be useful in formulating of species-specific diluting media (extenders) and improve cryopreservation techniques used for long term conservation of fish sperm. In Persian sturgeon, a significant positive relationship was observed between the duration of sperm movement and K^+ concentration of the seminal plasma. The close correlation between the levels of ionic and sperm motility shows a regulatory function for the ionic composition (especially K^+ ion) during spermiation of Persian sturgeon. This result was also in agreement with results reported by Bozkurt et al. (2008) (14) in Grass carp and Hajirezaee et al. (2009) (20) in Caspian brown trout, *Salmo trutta caspius*. We also observed a significant relationship between sperm motility and total protein concentration. In agreement with our result, similar correlation was found by Hajirezaee et al. (2009) (20) in the Caspian brown trout. Lahnsteiner et al. (2004) (27) reported that seminal plasma proteins prolong the viability of rainbow trout sperm. Analysis of the seminal plasma proteins provides new insights into sperm motility and fertilizing abilities, thereby creating possibilities for improving propagation programs (e.g. gamete conservation technologies) (30). These researches shown that some seminal plasma proteins have a key role in the motility of sperm cells, but additional studies are needed to define the types of proteins affecting sperm motility and the mechanism of the reactions.

Conclusion

Study of seminal plasma characteristics and their relationships with sperm motility could be useful in order to developing a special extenders and protectant solution for improvement of artificial fertilization and cryopreservation of Persian sturgeon sperm. In this regard, K^+ ion and total protein of seminal plasma may be as key indicators of sperm quality of Persian sturgeon due to their close relationships with sperm motility.

Acknowledgments

This work was supported by Tarbait Modares University. The authors wish to thank the staff of the Rajaei Sturgeon Hatchery Center, Sari, Iran for providing the broodfish for this research.

References

- Alavi, S.M.H., Cosson, J., Karami, M., Abdoulhay, H., & Amiri, B.M. (2004). Chemical composition and osmolality of seminal fluid of *Acipenser persicus*; their physiological relationship with sperm motility, *Aqua Res*, 35, 1238-1243.
- Alavi, S.M.H., Cosson, J., Karami, M., Amiri, B.M., & 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., & Cosson, J. (2006) Sperm motility in fishes: (II) effects of ions and osmotic pressure. *Cell Biology International*, 30, 1-14.
- Alavi, S.M.H., Cosson, J., & Kazemi, R. (2006). Semen characteristics in *Acipenser persicus* in relation to sequential stripping. *Journal of Applied Ichthyology*, 22, 400-405.
- Alavi, S.M.H., Psenicka, M., Rodina, M., Policar, T., & Linahart, O. (2008). Changes of sperm morphology, volume, density and motility and seminal plasma composition in *Barbus barbus* (Teleostei: Cyprinidae) during the reproductive season. *Aquat. Living Resour*, 21, 75-80.
- Aramli, M.S., Kalbassi, M.R., & Nazari, R.M. (2013). Study of sperm concentration, seminal plasma composition and their physiological correlation in the Persian sturgeon, *Acipenser persicus*. *Reproduction in Domestic Animals*, doi: 10.1111/rda.12207.
- Babaei, S.S., Abedian Kenari, A., Nazari, R.M., & Gisbert, E. (2011). Developmental changes of digestive enzymes in Persian sturgeon (*Acipenser persicus*) during larval ontogeny. *Aquaculture* 318, 138-144.
- Beer-Ljubic, B., Aladrovic, J., Marenjak, T.S., Laskaj, R., Majic-Balic, I., & Milinkovic-Tur, S. (2009). Cholesterol concentration in seminal plasma as a predictive tool for quality semen evaluation. *Theriogenology*, 72, 1132-1140.
- Bemis, W., & Kynard, B. (1997). Sturgeon Rivers: an introduction to Acipenseriform biogeography and life history. *Environ Biol Fishes*, 48, 167-183.
- Billard, R. (1986). Spermatogenesis and spermatology of some teleost fish species. *Reproduction Nutrition Development*, 26, 877-920.
- Billard, R., Cosson, J., Crim, L.W., & Suquet, M. (1995). Sperm physiology and quality. In: Bromage, N.R., Roberts, R.J. (eds) *Brood Stock Management and Egg and Larval Quality*, Blackwell Science, Oxford, pp, 25-52.
- Billard, R., Cosson, J., Fierville, F., Brun, R., Rouault, T., & Williot, P. (1999). Motility analysis and energetics of the Siberian sturgeon, *Acipenser baerii* spermatozoa. *Journal of Applied Ichthyology*, 15, 199-203.
- Birstein, V.J., Hanner, R., & DeSalle, R. (1997). Phylogeny of the Acipenseriformes: cytogenetic and molecular approaches. *Environmental Biology of Fishes*, 48, 127-155.
- Bozkurt, Y., Ogretmen, F., Erein, U., & Yidz, M. (2008). Seminal plasma composition and its relationship with physical spermatological parameters of Grass carp (*Ctenopharyngodon idella*) semen: with emphasis on sperm motility. *Aquaculture Research*, 39, 1666-1672.
- Bozkurt, Y., Ogretmen, F., Kokcu, O., & Ercin, U. (2011). Relationships between seminal plasma composition and sperm quality parameters of the *Salmo trutta macrostigma* (Dumeril, 1858) semen: with emphasis on sperm motility. *Czech J Anim Sci*, 56, 355-364.
- Ciereszko, A., Glogowski, J., & Dabrowski, K. (2000). Biochemical characteristics of seminal plasma and spermatozoa of freshwater fishes. In: Tiersch, T.R., Mazik, P.M. (eds) *Cryopreservation of Aquatic Species*, World Aquaculture Society, Baton Rouge, LA, USA, pp, 20-48.
- Ciereszko, A. (2008). Chemical composition of seminal plasma and its physiological relationship with sperm motility, fertilizing capacity and cryopreservation success in fish. In: Alavi,

- S.M.H., Cosson, J., Coward, K., Rafiee, G.R. (eds) Fish spermatology. Alpha Science Ltd, Oxford, pp, 215-240.
18. Drabkina, B.M. (1961). Effect of different water salinities on the survival rate of spermatozoa, eggs and larvae of sturgeon. Doklady Academy NAUK SSSR, 138: 492-495.
19. Gallis, J.L., Fedrigo, E., Jatteau, P., Bonpunt, E., & Billard, R. (1991). Siberian sturgeon spermatozoa: effects of dilution, pH, osmotic pressure, sodium and potassium ions on motility. In: Williot P (ed), Acipenser . Cemagref Publication, Antony, France, pp, 143-151.
20. Hajirezaee, S., Mojazi Amiri, B., & Mirvaghefi, A.R. (2009). Effect of stripping frequency on semen quality of endangered caspian Brown trout (*Salmo trutta caspius*). American Journal of Animal and Veterinary Science, 4(3), 65-71.
21. Haxton, T.J. (2006). Characteristics of a Lake sturgeon spawning population sampled a half century apart. J Gt Lakes Res, 32, 124-130.
22. Hwang, P.C., & Idler, D.R. (1969). A study of major cations, osmotic pressure, and pH in seminal components of Atlantic salmon. Journal of the Fisheries Research Board of Canada, 26, 413-419.
23. Kruger, J.C., Smith, G.L., Van Vuren, J.H.J., & Ferreira, J.T. (1984). Some chemical and physical characteristics of the semen of *Cyprinus carpio* and *Oreochromis mossambicus*. Journal of Fish Biology, 24, 263-272.
24. Lahnsteiner, F., Patzner, R.A., & Weismann T. (1993). Energy resources of spermatozoa of the Rainbow trout, *Oncorhynchus mykiss* (Pisces, Teleostei). Reproduction Nutrition Development, 33, 349-360.
25. Lahnsteiner, F., Berger, B., Weismann, T., & 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.
26. Lahnsteiner, F., Berger, B., Weismann, T., & 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.
27. Lahnsteiner, F., Mansour, N., & Berger, B. (2004). Seminal plasma proteins prolong the viability of Rainbow trout (*Oncorhynchus mykiss*) spermatozoa. Theriogenology, 62, 801-808.
28. Linhart, O., Slechta, V., & Slavik, T. (1991). Fish sperm composition and biochemistry. Bulletin of the institute of zoology. Academia Sinica, Monograph16, 285-311.
29. Linhart, O., Rodina, M., Gela, D., & Kocour, M. (2004). Optimization of artificial propagation in European catfish, *Silurus glanis* L., Aquaculture, 235: 619-632.
30. Li, p., Hulak, M., & Linhart, O. (2009). Sperm proteins in teleostean and chondrosteian (sturgeon) fishes. Fish Physiology and Biochemistry, 35(4), 567-581.
31. Li, P., Rodina, M., Hulak, M., Li, Z.H., & Linhart, O. (2011). Spermatozoa concentration, seminal plasma composition and their physiological relationship in the endangered Stellate sturgeon (*Acipenser stellatus*) and Russian sturgeon (*Acipenser gueldenstaedtii*). Reproduction in Domestic Animal, 46(2), 247-252.
32. Moghim, M., Vajhi, A.R., Veshkini, A., & Masoudifard, M. (2002). Determination of sex and maturity in *Acipenser stellatus* by using ultrasonography. Journal of Applied Ichthyology, 18, 325-328.
33. Morisawa, M., Hirano, T., & Suzuki, K. (1979). Changes in blood and seminal plasma composition of the mature salmon, *O. keta*, during adaptation. Comparative Biochemistry and Physiology, 64, 325-329.
34. Nazari, R.M., Modanloo, M., Ghomi, M.R., & Ovissipor, M.R. (2010). Application of synthetic hormone LHRH-A₂ on the artificial propagation of Persian sturgeon *Acipenser persicus*. Aquaculture International, 18, 837-841.
35. Perchec, G., Cosson, J., Andre, F., & Billard, R. (1995). Degradation of the quality of Carp sperm by urine contamination during stripping. Aquaculture, 129, 133-137.
36. Piironen, J., & Hyvarinen, H. (1983). Composition of the milt of some teleost fishes. Journal of Fish Biology, 22, 351-361.
37. Piironen, J. (1994). Composition and cryopreservation of sperm from some Finnish freshwater teleost fish. Finnish Fisheries Research, 15, 27-48.
38. Piros, B., Glogowski, J., Kolman, R., Rzemieniecki, A., Domagala, J., Horvath, A., Urbanyi, B., & Ciereszko, A. (2002). Biochemical characterization of Siberian sturgeon *Acipenser baeri* and starlet, *Acipenser*

39. *ruthenus*, milt plasma and spermatozoa. Fish Physiology and Biochemistry, 26, 289-295.
40. Psenicka, M., Alavi, S.M.H., Rodina, M., Cicova, Z., Gela, D., Cosson, J., Nebesarova, J., & Linhart, O. (2008). Morphology, chemical contents and physiology of chondrosteian fish sperm: A comparative study between Siberian sturgeon (*Acipenser baerii*) and sterlet (*Acipenser ruthenus*). Journal Applied Ichthyology, 24, 371-377.
41. Redondo-Muller, C., Cosson, M.P., Cosson, J., & Billard, R. (1991). In vitro maturation of the potential for movement of carp spermatozoa. Molecular Reproduction Development, 29, 259-70.
42. Rochard, E., Castelnaud, G., & LePage, M. (1990). Sturgeons (Pisces: Acipenseridae); threats and prospects. J Fish Biol, 37, 123-132.
43. Rosengrave, P., Taylor, H., Montgomerie, R., Metcalf, V., McBride, K., & Gemmell, N.J. (2009). Chemical composition of seminal and ovarian fluids of Chinook salmon (*Oncorhynchus tshawytscha*) and their effects on sperm motility traits. comp biochem physiology, 152, 123-129.
44. Rurangwa, E., Kime, D.E., Ollevier, F., & Nash, J.P. (2004). The measurement of sperm motility and factors affecting sperm quality in cultured fish. Aquaculture, 234, 1-28.
45. Secer, S., Tekin, N., Bozkurt, Y., Bukan, N., & Akcay, E. (2004). Correlation between biochemical and spermatological parameters in Rainbow trout (*Oncorhynchus mykiss*) semen The Israeli Journal of Aquaculture-Bamidgeh, 56, 274-280.
46. Secer, S., Tekin, N., Bozkurt, Y., Bukan, N., & Akcay, E. (2004). Correlation between biochemical and spermatological parameters in Rainbow trout (*Oncorhynchus mykiss*) semen The Israeli Journal of Aquaculture-Bamidgeh, 56, 274-280.
47. Soengas, J.L., Sanmartin, B., Barciela, P., Aldegunde, M., & Rozas, G. (1993). Changes in carbohydrate metabolism in domesticated Rainbow trout *Oncorhynchus mykiss* related to spermatogenesis. Comparative Biochemistry and Physiology, 105, 665-671
48. Stoss, J. (1983). Fish gamete preservation and spermatozoan physiology. Fish Physiology, 9, 305-350.
49. Toth, G.P., Ciereszko, A., Christ, S.A., & Dabrowski, K. (1997). Objective analysis of sperm motility in the Lake sturgeon, *Acipenser fulvescens*: activation and inhibition conditions. Aquaculture, 154, 337-348.
50. Tsvetkova, L.I., Cosson, J., Linhart, O., & Billard, R. (1996). Motility and fertilizing capacity of fresh and frozen-thawed spermatozoa in sturgeons *Acipenser baerii* and *Acipenser ruthenus*. Journal of Applied Ichthyology, 12, 107-112.