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Quantitive characteristics and chemical composition in Caspian Roach (*Rutilus rutilus caspicus*) sperm

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Abstract

In this study, quantitive characteristics and chemical composition of in roach (*Rutilus rutilus caspicus*) sperm were investigated. Sperm traits included sperm movement duration, percentage of motile spermatozoa, sperm density, spermatocrit and sperm volume. Some seminal plasma characteristics (sodium, potassium, calcium, magnesium and chloride) were investigated. In addition, some metabolites of seminal plasma (glucose, cholesterol and protein) were measured. The Na^+ and K^+ ions correlated negatively with spermatozoa motility (r = -0.0518, $p<.05$ and r =-0.3597, $p<.01$) respectively. Also, there were significant positive correlations between Ca^{2+} and Cl^- ions with spermatozoa motility (r = 0.2945, $p<.05$ and r= 0.1379, $p<.01$), respectively. Mg^{+2} was positively correlated with glucose and protein (r = 0.046, $p<.05$ and r = 0.694, $p<.05$), respectively. On the other hand, a significant positive relationship was found between Na^+ and K^+ (r = 0.548, $p<.01$). These parameters can be used to evaluation of sperm quality and collecting information about developing procedures for artificial fertilization of roach.

Keywords: Sperm motility, Seminal plasma, Sperm quality, Roach

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Introduction

The roach, *Rutilus rutilus caspicus* (Teleostei: Cyprinidae) is a freshwater species that contributed to fishery at southern Caspian Sea (Abdoli, 2000). The roach is a migratory fish that spawned in the river of Atrak and other rivers in Iran such as Gharesoo and Gorganrood. Reproductive migration in the southern Caspian Sea starts in February and continues until the spring Petr (1987). Most migration time take place in 10 to 12 water temperature (Petr, 1987). The average maturation age of this fish is 3-4 yr . Male fish on average one year earlier than females become mature (Petr, 1987). Proper management of male broodstock is a factor that influences gamete quality and artificial fertilization (Billard et al., 1995). The fertilization success depend on many factors such as the condition of male reproductive and the quality and quantity of sperm such as density, motility, movement duration and seminal plasma composition (Billard et al., 1995). Several factors has been documented to evaluate the sperm quality including motility, spermatocrit, spermatozoa concentration, fertilizing capacity, osmolality and pH of seminal plasma, chemical composition of seminal plasma and several others (Rurangwa et al., 2004). Sperm motility is a key factor that determines fertilization success (Billard et al., 1995). Determination of sperm quality parameters such as seminal plasma composition, sperm production characteristics and sperm motility traits could help us to develop and improve artificial reproduction in fish farms (Alai et al., 2008). Better knowledge about sperm biochemistry and the mechanism of reproduction are important to improving artificial fertilization. In male brood fish, high quality of semen is essential for increasing the efficiency of artificial fertilization. There are many factors contributing to evaluation of sperm quality such as rearing condition, sperm collection, and storage of semen and sperm activation conditions. An analysis of sperm characteristic provides a reasonable basis for developing a strategy for maximizing the fertility of a fish. Sperm consist of seminal plasma and spermatozoa. Seminal plasma contains substances that support sperm cell. Some substances reflect the functioning of the reproductive system and spermatozoa (Ciereszko et al., 2000). The main role of seminal plasma is to create an optimal condition for spermatozoa storage. In addition, seminal plasma has beneficial functions for spermatozoa during external fertilization by creating favorable micr-oenvironment for sperm movement (Billard, 1986; Ciereszko et al., 2000). Also, information about composition seminal plasma and other biological fluids can be used to make media for use as a diluent or for gamete storage. Correlation between composition of the seminal plasma and motility of spermatozoa has been investigated only a few species, *Salmo salar* (Hwang and Idler, 1969), *Alburnus alburnus* (Kruger et al., 1984), *Cyprinus carpio* and *Oreochromis mossambicus* (Lahnsteiner et al., 1996), *Acipenser baeri* and *Acipenser fluvescence*

(Galis et al., 1991) and *Ctenopharyngdon idella* (Buzkurt et al., 2009). Objectives of this study were to determine the sperm characteristics in roach semen, as well as effects of seminal plasma characteristics on sperm motility were investigated.

Materials and methods

Male Broodstock were caught from Gorganrood River in Golestan province during spawning season (February to April). Water temperature was ranged between 11 to 12ºC during breeding season. Sperm was collected from 30 mature males (weight = 52.21 ± 12.67 g, total length= 17.77 ± 1.19 cm) by manual abdominal stripping. Semen was stored into glass tube separately for each fish and held on dry ice (4ºC) until measurement. An activating solution of 0.3% NaCl was used for estimating motility. For the evaluation of motility, about 1µL of semen was placed on a glass microscope slide and 100µL of activation solution was added. Motility was recorded using a camera (Panasonic wv.cp240 Japan) mounted on a phase contrast microscope (Leica USA). The duration of sperm motility was measured immediately after initiation of sperm activation until 100% spermatozoa were immotile and expressed as sperm movement duration. Sperm motility was defined as the percentage of progressively motile spermatozoa within each activated sample. Progressively motile spermatozoa were defined as those actively swimming in a forward motion. Only forward moving sperm was judged as motile and sperm cells that vibrated in place were not considered motile (Aas et al., 1991). Observations were made within two hours of semen collection. The spermatocrit was defined as the ratio of volume of white packed material to the total volume of semen ×100 (4). Semen was drawn into glass microhaematocrit capillary tubes (75mm length, 1-1–1-2mm internal diameter) until 60–80% of the tube volume was occupied by semen. One end of the tube sealed with clay and then tubes were centrifuged for 8 min at 3000 rpm (Sigma, 13 USA). Spermatozoa density was determined by counting. Spermatozoa concentration was calculated with haemocytometer by placing a droplet of diluted semen with 0.3% NaCl solution on a Thomas haemocytometer slide (depth 0.1mm) with a cover slip and counted using light microscopy. After a few minutes (to allow sperm sedimentation), the number of spermatozoa was counted at 100X magnification and expressed as spermatozoa $\times 10^9$ per ml. Measurements were taken in triplicate for each sample and average of the three measurements was used for the results. Semen volume was measure in scaled tubes and expressed as (ml). Sperm samples were centrifuged at 3000 rpm for 8 min (Eppendorf AG, Hamburg, Germany) and then seminal plasma (supernatant) was collected. Plasma centrifuged twice to avoid possible contamination with spermatozoa. The pH was measured using pH indicator strip (pH meter 713 Herisau Switzerland). The plasma was frozen at -20 ºC until analysis. Ca^{+2} and Mg^{+2} of the seminal plasma were measured spectrophotometrically (S2000-UV/VIS,

England). The concentration of Na^+ and K^+ were determined with flame photometer (Jenway PFP, England; Standard kits from Parsazmoon, Iran). Data from individual fish on all sperm parameters were analyzed using one-way analysis of variance (ANOVA). Significant means were subjected to a multiple comparison test (Duncan) at α = 0.05 level. Before analysis, data were tested for normality and homogeneity of variance. Correlation between spermatological parameters and seminal plasma composition were estimated using Pearson correlation test. Results are presented as mean ± SEM. The SPSS software was used for data analysis.

Results

The sperm traits are given in Table 1. No significant relationship was found between sperm volume and sperm traits, but there was a positive relationship between Mg^{+2} and glucose ($p>.05$). A significant positive correlation was observed between sperm motility and density (r=0.0077, $p<.05$), also spermatozoa motility correlated positively with pH (r = 0.2945, $p<.05$). On the other hand, spermatozoa motility correlated negatively with Na^+ (r = - 0.0518, $p>.05$) and K^+ (r=-0.3597, P>0.01). Spermatocrit also correlated significantly with Ca^{2+} and Cl^- (r=0.2899, $p<.05$ and r=0.1573, $p<.05$). A negative correlation was found between motility duration with Na^+ and K^+ ($p>.05$). The mineral and organic compositions of the seminal plasma are shown in Table 2. The following correlations were observed between mineral and organic components: The Na^+ and k^+ ions correlated negatively with spermatozoa motility (r =- 0.0518 $p>.05$ and r = - 0.3597, $p>.01$) respectively (Fig. 1 a, b). Mg^{+2} and Ca^{+2} ions correlated positively with spermatozoa motility (r = 0.0184, $p<.05$ and r = 0.2945, $p<.01$) respectively (Fig. 1, c, d). Also, a positive correlation was found between spermatozoa motility and Cl^- (r =0.1379, $p<.05$) (Fig. 1 e). The relationships between biochemical and spermatological parameters are shown in Table 3. Spermatozoa motility correlated negatively with metabolite composition of the seminal plasma ($p>.05$) (Fig. 1f, g and h). The Cl^- ion correlated positively with Cholesterol (r=0.039, $p<.01$). On the other hand, a significantly positive relationship was found between total protein and glucose (r=0043, $p<.01$). Highly significant relationship was detected between Na^+ and K^+ (r = 0.3003, $p<.01$).

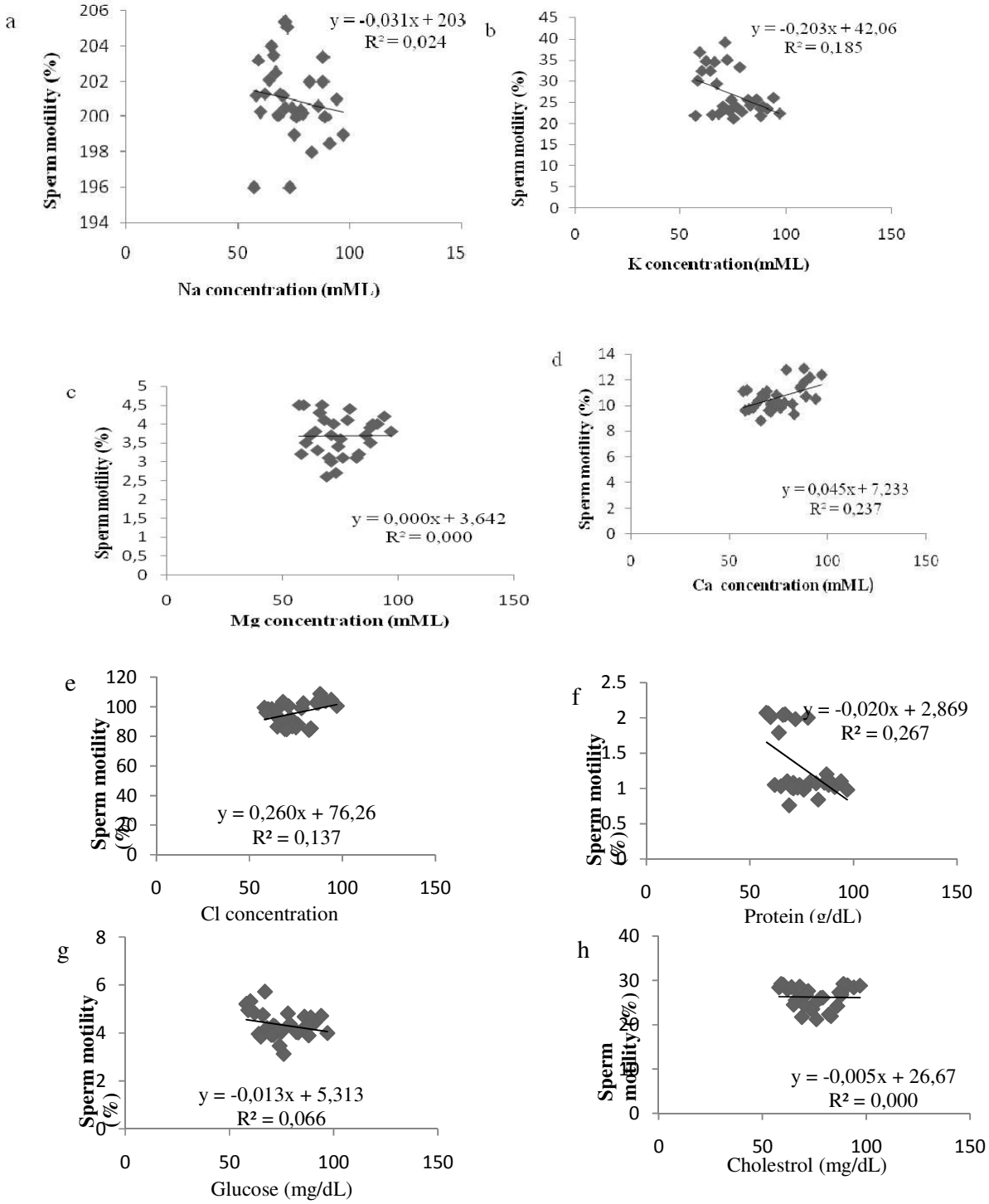

Figure 1: The relationships between sperm motility and Na^{+} (a), K^{+} (b), Ca^{+2}(c), Mg^{+2}(d), Cl^{-}(e), total protein (f), glucose (g), cholesterol (h). (*p>.05*, ANOVA).

Table 1: Spermatological parameters of Roach

parameters	minimum	maximum	mean	SEM
Volume(ml)	0.20	0.50	0.35	0.14
Sperm motility (%)	57	97	76.2	11.28
Motility duration (s)	32	62	52.68	5.71
Sperm density ($\times 10^9$)	1.25	1.50	1.37	0.06
Spermatocrit (%)	52	72	61.16	5.71
pH	7.40	8	7.95	0.55

Table 2: Seminal plasma ions and metabolites composition of Roach

variables	Minimum	Maximum	Mean	SEM
Na^+ (mM/ml)	197	204	200.94	87.84
K^+ (mM/ml)	21	33	26.91	5.32
CL^- (mM/ml)	84.30	108.70	95.87	7.69
Ca^{+2} (mM/ml)	9.84	11.64	10.95	7.69
Mg^{+2} (mM/ml)	2.19	4.50	3.95	0.64
Glucose (mg/dl)	4.21	5.72	4.34	0.55
Protein (mg/dl)	0.76	2.05	1.29	0.44
Cholesterol ($mgdL^{-1}$)	24.27	28.98	26.43	1.64

Table 3: Correlation between spermatological parameters and seminal plasma Composition of Roach

	Motility	spermatocrit	Density	Na^+	CL-	Ca^{+2}	Mg^{+2}	Glucose	Protein
Movement duration	0.054								
Density	0.415*	-0.088							
pH	0.343*	-0.223	0.082						
Na^+	-0.358*	-0.228	0.170	-0.088					
K^+	-0.633**	0.026	-0.066	0.548**	0.135				
CL^-	0.439*	0.412*	0.238	0.033	0.126	0.178			
Ca^{+2}	0.580**	0.420*	0.379*	-0.99	0.571	0.051	0.028		
Mg^{+2}	0.110*	0.103	0.172	0.233	0.536	0.338	0.571	0.086	
Glucose	-0.098	-0.109	-0.665	0.177	0.469	-0.068	0.046*	0.197	0.333**
Protein	-0.531	-0.046	-0.217	0.367	0.163	-0.275	0.043	0.467	0.362
Cholesterol	- 0.026	-0.517	0.119	0.647	0.393**	0.737	0.246	0.382	0.047**

* Significant at $p<.05$.
**Significant at $p<.01$

Discussion

Motility duration was differed from results reported by Akcay et al. (2004), Buzkurt et al. (2005) for Mirror carp. Most studies on fish species have shown that the duration and motility of semen may vary seasonally (Benau and Terner, 1980). The differences may be due to feeding conditions, age, environmental factors, and time of spawning or dilution ratio. In present study, mean sperm motility and motility duration was determine as 76.2 ± 11.28% and 52.68 ± 5.71 sec, respectively which is disagree with results reported for grass carp (Bozkurt et al., 2009). The composition of seminal plasma has a great influence on the biological quality of the milt and these factors are directly related to the fertilization success (Rurangwa et al., 2004; Alavi et al., 2005). Chemical criteria importances are the presence or absence of inorganic and organic component in the semen. This may be also lead to better understanding of the mechanisms of fertilization. Fish seminal plasma, in compare to that the of higher vertebrates is characterized by a low total protein concentration, substantial mineral compounds (Na^+, K^+, Cl^-, Ca^{+2}, Mg^{+2}) and low concentration of organic substances. Depending on ionic concentration, most of these ions are involved in regulating sperm motility either by contributing to the intracellular ionic composition by regulating osmolality (Billard et al., 1992). Seminal plasma of roach has a higher Na^+ content than common carp 75 mMl^{-1}(Morisawa et al., 1983), Perch 124 mMl^{-1} (Lahnsteiner et al., 1995), Catfish 164 mMl^{-1} (Tan-Fermin et al., 1999) and common barbel 74 mMl^{-1} (Alavi et al., 2008). However, the K^+ content of seminal plasma was lower than those report for common carp 70 mMl^{-1} (Aas et al., 1991) and common barbel 85 mMl^{-1} (Alavi et al., 2008), but higher than Perch 10 mMl^{-1} (Lahnsteiner et al., 1995) and catfish 18mMl^{-1} (Tan-Fermin et al., 1999). These differences probably represent species-specific characteristics (Ciereszko et al., 2000). Electrolytes (especially Na^+ and Cl^-) make certain the viability of sperm. The K^+ ion has role in keeping spermatozoa in the quiescent state (Baynes et al., 1981). Low levels of Na^+ and K^+ ion are associated with low percentages of motile spermatozoa and such semen should be low quality. Sperm motility of roach spermatozoa are observed to increase when the Na^+ and K^+ ion levels decrease and Ca^{+2} ion level increase in the seminal plasma (Figure 1 a, b and c). The findings of this study indicated that Ca^{+2} and Mg^{+2} increased the motility of roach sperm. Buzkurt et al. (2009) observed a similar finding with scaly carp sperm, but differed from results reported by Billard and Cosson (1992). The content of Ca^{2+} in the seminal plasma significantly correlated with the semen fertilization capacity. Further, Ca^{+2} and Mg^{+2} contributed significantly to the ionic composition of seminal plasma (Secer et al., 2004). The specific role of protein in fish semen is unknown. White and Macleod (1963) indicated that protein had a protective role. Also, Li et al. (2008) reported sperm proteins in the seminal plasma and spermatozoa of teleostean and chondrostean have evolved

adaptation due to the change in water environment. In this study, concentrations of total protein were found as 1.29 ± 0.44 mg/dl. The positive relationship of the protein with Mg^{+2} and cholesterol can be considered to be effective on sperm motility. However, Lahnsteiner et al. (2004) found that seminal plasma proteins prolong the viability of rainbow trout spermatozoa as measured by sperm motility. Fish spermatozoa are capable of utilizing extracellular carbohydrates. In this study, glucose has been identified in the seminal plasma and its concentration was found to be 4.34 ± 0.55 mg/dl. Glucose is the main sugar in the seminal plasma. Importance of glucose in fish semen is unclear. On the other hand, the presence this carbohydrate in seminal plasma has been connected to the high energy demand for the testes during spermatogenesis or to lipid synthesis of spermatozoa (Soengas et al., 1993). Various lipid classes have been found in seminal plasma and their level are vary greatly among fish species, such as 0.37 mg/dl for grass carp (Bozkurt et al., 2009) and 1 mg/dl for Eurasian Perch (Piironen and Hyvarinen 1983). According to Piironen (1994) seminal plasma lipid is associated with metabolism in spermatozoa. In our experiment, mean level of cholesterol positively correlated with sperm motility. There is insufficient information about the role of cholesterol in seminal plasma in spite of its identification in the seminal plasma of freshwater fish (Billard et al., 1995). Lipids and cholesterol may have a protective effect against environmental changes (especially in temperature) that occurs when the fish semen is released (Bozkurt et al., 2009). The knowledge of quantitive characteristics and chemical composition of sperm is a prerequisite for the successful evaluation of the reproductive ability of different fish species. This may also lead to the better understanding of fertilization mechanisms. On the other hand, there are some species-specific characteristics in terms of the mineral and organic composition of seminal plasma that should be considered for artificial insemination or sperm storage. It can be concluded that the findings of this research can be used to select high-quality mature males for egg fertilization in a commercial aquaculture operation and, as a result of reducing the number of male broodstock, the economic efficiency of the farm can be increased. The information on quantitive characteristics and chemical composition obtained in the present study could lead to more efficient gamete management and increase yields, and enhance the suitability of semen for short term storage.

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