

Correlation between chemical composition of seminal plasma and sperm motility characteristics of Prussian carp (*Carassius gibelio*)

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Abstract. The objectives of the present study were to determine the relationships between chemicals compositions of seminal plasma with sperm motility traits in Prussian carp, *Carassius gibelio* (Bloch, 1782). There were significant positive correlations between sperm movement duration and Ca^{+2} of semen. Also, a significant positive relationship was found between percentage of motile spermatozoa and Ca^{+2} of semen. On the other hand, Na^{+} , Cl^{-} and pH correlated negatively with sperm movement duration. Understanding of such correlations can be useful to evaluation of sperm quality and make media (extender) for dilution of semen and improving sperm motility parameters of Prussian carp.

Key Words: sperm motility trait, chemical composition, seminal plasma, Prussian carp.

چکیده: مطالعه حاضر با هدف تعیین ارتباط بین ترکیبات شیمیایی مایع سمینال اسپرمی با صفات حرکتی اسپرم ماهی قرمز *Carassius gibelio* (Bloch, 1782) انجام پذیرفت. همبستگی مثبت معنی داری بین طول مدت تحرک اسپرم و کلسیم موجود در مایع سمینال مشاهده شد. همچنین، مشخص شد که رابطه مثبت معناداری میان درصد تحرک اسپرم و میزان کلسیم پلاسمای اسپرمی وجود دارد. از سوی دیگر، سدیم، کلر و pH ارتباط منفی با طول مدت حرکت اسپرم داشتند. شناخت چنین روابطی می تواند در جهت ارزیابی کیفیت اسپرم و ساخت اکستندر ها برای رفیق کردن مایع منی و بهبود پرامترهای حرکتی اسپرم ماهی قرمز مفید باشد.
کلمات کلیدی: ویژگی های حرکتی اسپرم، ترکیبات شیمیایی، پلاسمای سمینال، ماهی قرمز

Introduction. *Carassius* complex can be considered as the most well known fish in history (Balon 2004). Today there is no other ornamental fish so popular and easy to obtain as goldfish. Due to its easy availability and hardiness, goldfish became one of the most commonly used laboratory animals. Many scientific studies, especially in the field of physiology, used *Carassius* sp. as animal model (Rylkova et al 2010). Besides, *Carassius* complex has an economical relevance (e.g. *Carassius gibelio*, sport fishing and/or pisciculture of subsistence in many countries).

The use of high quality gametes from captive fish broodstock is of great importance for ensuring the production of valuable offspring for aquaculture (Kjørsvik et al 1990; Bromage & Roberts 1995). Sperm quality of male broodstock affects the production of healthy larvae. Seminal plasma produced by the sperm duct provides an ionic environment that maintains the viability of spermatozoa after their release from the testes (Ciereszko 2008). Various factors can affect on sperm motility such as pH, temperature, ions and osmolality (Alavi & Cosson 2006). Because, high quality of semen is important to the fisheries industry and laboratory research, the biochemical composition of teleost semen has been studied by many researchers over the years (Piironen & Hyvarinen 1983; Billard & Menezo 1984). Some parameters such as spermatocrit, sperm density, fertilization capacity, pH, osmolality and seminal plasma composition are used to evaluation of sperm quality (Billard et al 1995). The seminal plasma analysis includes inorganic constituents (Na^{+} , K^{+} , Ca^{2+} , Mg^{2+}) involved in the process of inhibition or activation of sperm motility (Morisawa et al 1983; Morisawa 1985). Relationship between seminal plasma composition and sperm motility have been documented in some species; Atlantic salmon, *Salmo salar* (Hwang & Idler 1969), Common carp, *Cyprinus carpio* (Kruger et al 1984), bleak, *Alburnus alburnus* (Lahnsteiner et al 1996), Rainbow trout, *Oncorhynchus mykiss* (Lahnsteiner et al 1998),

Persian sturgeon, *Acipenser persicus* (Alavi et al 2004), Chinook salmon, *Oncorhynchus tshawytscha* (Rosengrave et al 2009). Fish spermatozoa are immotile in seminal fluid because of its chemical properties. In this paper, value of chemical parameters of semen including the inorganic composition (Na^+ , K^+ , Ca^{2+} , Mg^{2+} Cl^-), organic composition (protein, glucose and cholesterol) and pH and their relationships with sperm motility characteristics (percentage and duration of motility) were investigated in *Prussian carp*.

Material and Method. The experiment was carried out at the aquaculture Center of Gorgan University, Iran. Males were captured from reared hatchery at Nahar Khoran, Gorgan, during the spawning season of *Prussian carp*. To stimulate fish for spawning we injected intraperitoneally: 0.5 ml kg^{-1} b.w. *Ovaprim* (sGnRHa+dompridon). Milt samples were collected during the 2010 spawning season from 30 sexually mature two-year-old male Prussian carps (TL: 60.9 ± 5.3 cm, TW: 1572.7 ± 177.9 g). Semen samples were collected by massage from the anterior portion of the testis towards the genital papilla. Care was taken to avoid contamination of the semen with water, mucus, blood cells, faeces or urine. To analyse the ionic composition of seminal plasma, the semen was separated from the seminal plasma by centrifugation (Eppendorf AG, Hamburg, Germany) and the supernatant was separated and stored frozen at -20 °C until the time of analysis. The pH of seminal plasma was immediately determined using a laboratory pH meter (pH meter, Iran 762). Two mineral (Ca^{+2} and Mg^{+2}) and three biochemical parameters (total protein, glucose and cholesterol) of the seminal plasma were measured by spectrophotometric method (S2000-UV/VIS England). The concentration of Na^+ and K^+ were determined with flame photometer (Jenway PFP, England) (standard kits from Parsazmoon, Tehran, Iran).

Sperm motility analysis. Sperm motility triggered directly in activation medium 0.3% NaCl at ratio 1:1000 and immediately recorded by a videocamera (Panasonic wv.cp240 Japan) coupled with dark field 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. Percentages of motile spermatozoa after activation (%) were measured. Only forward moving sperm were judged motile, those simply vibrating or turning on their axes was considered immotile (Aas et al 1991).

Statistical analysis. The relationship between composition of the seminal fluid and sperm motility characteristic (sperm movement duration) was tested using the bivariate correlation coefficients of Pearson. Then, the Linear and non-linear regression models were investigated using regression fits. The sperm movement duration was used as dependent and the parameters of seminal fluid as independent variables.

Results and Discussion. The maximum, minimum, and mean of the percentage (7s after activation) and duration of motility, ion composition and pH of the seminal fluid of Prussian carp have been shown in Table 1.

Significant positive relationships were detected for the percentage of motile spermatozoa vs. Ca and also, the duration of motility vs. Ca^{+2} of semen (Table 2). A negative relationship was recorded between sperm movement duration and Na^+ , Cl^- and pH (see Table 2).

On the other hand, no relationship was found between metabolites of composition of seminal plasma (glucose, total protein and cholesterol) and sperm movement duration and percentage of motile spermatozoa. A negative correlation between percentage of motile spermatozoa and K^+ was recorded. Significant positive correlation between Ca^{+2} and sperm movement duration is shown in (Figure 1).

This is the first study that shows chemical composition of seminal plasma and its relationship with sperm motility characteristics in Prussian carp. The differences in the concentrations of inorganic components of semen samples of Prussian carp were observed.

Table 1

Maximum, minimum, and the mean of seminal plasma parameters and motility characteristics of sperm in Prussian carp

<i>Seminal plasma parameters</i>	<i>Maximum</i>	<i>Minimum</i>	<i>Mean</i>	<i>SD</i>
Sperm movment duration (s)	48.46	18.81	33.63	4.03
Percentage of motile spermatozoa	84	70	79	3
Na (mM.L)	173.18	99.69	101.59	21.29
K (mM.L)	32.18	20.22	26.20	3.84
Cl ⁻ (meqL)	163.56	122.8	143.18	11.77
Ca (mM.L)	0.98	0.34	0.66	0.17
Mg (mM.L)	2.02	1.04	1.53	0.28
pH	8.9	8.6	8.75	0.10
Glucose (mM l ⁻¹)	0.24	0.144	0.192	0.041
Total protein (gdl ⁻¹)	0.05	0.037	0.043	0.004
Cholestrol (mM l ⁻¹)	0. 15	0.016	0.083	0.037

Table 2

Relationships between the chemical composition of seminal plasma and sperm motility traits in Prussian carp

<i>Variables</i>	<i>Seminal plasma parameters</i>	<i>Bivariate coefficient</i>	<i>Regression function</i>	<i>R square</i>	<i>P value</i>
Sperm movment duration (Sec)	Na (mM.L)	- 0.443	$y = - 0.059x + 7.338$	0.413	≤ 0.05
	Cl ⁻ (meqL)	- 0.557	$y = - 0.025x + 8.384$	0.078	≤ 0.05
	pH	- 0.503	$y = - 36.27 + 0.085x + 6.553$	0.036	≤ 0.05
Percentage of motile spermatozoa	Ca (mM.L)	- 0.602	$y = 0.011x + 0.318$	0.36	≤ 0.05
	K (mM.L)	- 0.707	$y = - 0.642x + 73.82$	0.49	≤ 0.05

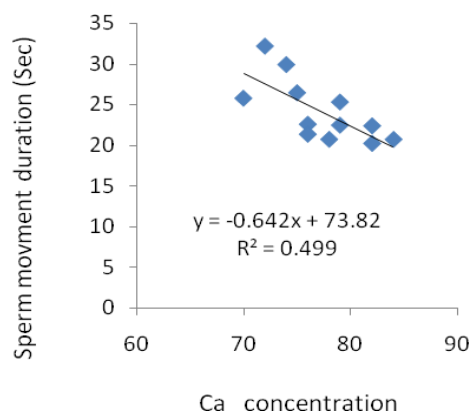


Figure 1. Relationship between the Ca⁺² and sperm movement duration in Prussian carp (independent variable: Ca⁺², dependent variable: sperm movment duration).

These differences may appear due to the differences in secretory activity of the spermatid duct epithelium of individuals since the formation of the seminal plasma in fish (inorganic as well as organic compounds) is a secretion process of the spermatid duct epithelium (Marshall 1986; Marshall et al 1989; Lahnsteiner et al 1994). In Prussian carp, significant positive relationships were observed between the duration of motility and percentage of motile spermatozoa with Ca^{+2} of semen respectively. In agreement with our results, similar relationships were found in other fish, for example, percentage of motile spermatozoa vs. Ca^{+2} in brown trout (Hajirezaee et al 2010). In contrast to our results, several authors were found that there was a negative relationship between the factors mentioned above; such results are that of Perez et al (2003) for European eel. Several studies have shown that presence of the organic and inorganic components supports the viability of spermatozoa (Morisawa et al 1983; Piironen & Hyvarinen 1983; Stoss 1983; Lahnsteiner et al 1994; Ciereszko et al 2000). In this regard, interactions of ions present in the seminal plasma with the sperm membrane do influence the membrane potential and represent a mechanism of inhibition of spermatozoa in the seminal plasma or sperm duct, allowing the maintenance of the potential of motility before release to the surrounding medium (Ciereszko et al 2000). These allow the maintenance of the potential of motility before release to the surrounding medium. In the present study, we found negative relationship between sperm movement duration and Na, Cl and pH of semen, whereas, some positive relationships were observed by several authors. For example, sperm motility vs. Na, K, pH in rainbow trout (Lahnsteiner et al 1998), sperm motility, sperm movement duration vs. Na, Mg, Cl, spermatozoa motility vs. Na, K, pH in *Alburnus alburnus* (Lahnsteiner et al 1996) and duration of motility vs. pH in the rainbow trout and chum salmon (*Oncorhynchus keta*) (Morisawa & Morisawa 1986; Morisawa & Morisawa 1988). But Rosengrave et al (2009) was not observed statistically significant correlations between sperm motility traits and compositions of seminal plasma. Also, Alavi et al (2004) observed no statistically significant correlations between seminal plasma composition and sperm motility traits for *Acipenser persicus*. In our study, a negative relationship was detected between percentage of motile spermatozoa and K^{+} of semen. Kusa (1950) demonstrated that a high potassium concentration in the seminal plasma of chum salmon (*O. keta*) inhibited sperm motility resulting in a decrease in fertilization success. In contrast to our results, Hajirezaee et al (2010) found a positive relationship between percentage of motile spermatozoa in brown trout. The effect of spermatozoa sensitivity to K^{+} in the seminal fluid may also vary through the reproductive season (Alavi & Cosson 2006). There appears to be considerable inter- and intra-specific variability in the ionic composition of seminal plasma in fish (Alavi & Cosson 2006). This may imply that different ions and ion concentrations are involved in regulating and initiating sperm motility for different fish species (Billard & Cosson 1992; Scott & Baynes 1980).

Conclusions. Highly significant relationships between percentage of motile spermatozoa with Ca^{+2} and also the sperm movement duration and Ca^{+2} suggest that this parameter could be considered the most important seminal plasma characteristics influencing the potential of motility of Prussian carp spermatozoa before sperm ejaculation. Thus, the Ca^{+2} and sperm movement duration of semen could be main indicators for evaluating of semen quality in Prussian carp.

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