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Seminal plasma composition and its relationship with physical spermatological parameters of Grass carp (*Ctenopharyngodon idella*) semen: with emphasis on sperm motility

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Abstract

In this research, the mineral and organic composition of the seminal plasma, physical spermatological parameters and their physiological relationships were investigated in grass carp (*Ctenopharyngodon idella*). The seminal plasma contained 98.14 ± 5.23 mM L^{-1} (Na^+), 380.85 ± 25.95 mM L^{-1} (K^+), 30.25 ± 4.96 mg dL^{-1} (Ca^{2+}), 19.16 ± 1.70 mEq L^{-1} (Mg^{2+}), 1.36 ± 0.11 mg dL^{-1} glucose, 0.37 ± 0.08 g dL^{-1} total protein, 12.02 ± 1.18 mg dL^{-1} cholesterol, 14.85 ± 1.50 mg dL^{-1} triglyceride and 43.5 ± 9.56 mg dL^{-1} urea. The following spermatological parameters were found: sperm volume 14.44 ± 1.16 mL, sperm motility 80.60 ± 1.55%, movement duration 67.68 ± 4.32 s, density 15.43 ± 0.72 × 10^9 mL^{-1}, total density 337.43 +45.86 × 10^9 and pH 7.24 ± 0.17. The Na^+ and Ca^{2+} ions correlated negatively with spermatozoa motility ($r = -0.453$, $P > 0.05$ and $r = -0.192$, $P > 0.05$) respectively. The K^+ ion correlated positively with spermatozoa motility ($r = 0.545$, $P > 0.05$). But a statistically significant correlation was not observed between sperm motility and seminal plasma parameters. The following correlations were observed between mineral and organic components. The Mg^{2+} was positively correlated with glucose and cholesterol ($r = 0.692$, $P < 0.05$ and $r = 0.680$, $P < 0.05$) respectively. A highly significant positive relationship was also found between Mg^{2+} and total protein ($r = 0.837$, $P < 0.01$). On the other hand, a significantly negative relationship was found between Ca^{2+} and triglyceride ($r = -0.639$, $P < 0.05$). These parameters should be considered when developing procedures for either artificial fertilization or for cryopreservation of grass carp sperm.

Keywords: sperm motility, seminal plasma, sperm quality, *Ctenopharyngodon idella*

Introduction

The proper management of male broodstock is a prerequisite for industrialized aquaculture farming. It is highly species specific and its success depends on many factors such as the food condition of the male reproductive accessory organs and the quality and quantity of sperm in terms of density, production, physiology and biochemistry (Billard, Cosson, Crim & Suquet 1995). An analysis of sperm characteristics provides a reasonable basis for developing a strategy for maximizing the fertility of a fish. Factors such as season, photoperiod, collection technique, temperature, time of collection, age and disturbances in spermatogenesis are well suited for studying variations in sperm characteristics (Baynes & Scott 1989; Cabrita, Anel & Herrae'z 2001; Asturiano, Perez, Garzon, Penaranda, Marco-Jimenez, Martinez Lorens, Tomas & Jover 2005). Sperm quality is an important factor in increasing the efficiency of artificial fertilization. Also, spermatoza motility is an important criterion for sperm quality, and is usually expressed as percentage and duration of motile sperm after activation (Lahnsteiner, Berger, Weismann & Patzner 1996).

Seminal plasma has a unique composition, containing substances supporting sperm cells and some

substances reflecting the function of the reproductive system and spermatozoa (Ciereszko & Dabrowski 2000). The main role of seminal plasma is to create an optimal environment for the storage of spermatozoa. In addition, seminal plasma has beneficial functions for spermatozoa during external fertilization by creating a favourable microenvironment for sperm movement (Billard 1986). It plays a crucial physioendocrinological role in supporting spermatozoa after the release of sperm from the testis into the sperm duct and subsequently after discharge of sperm into the aquatic environment (Morisawa & Morisawa 1988; Ciereszko, Glogowski & Dabrowski 2000; Alavi & Cosson 2006). Also, the composition of seminal plasma and other biological fluids can be used as a reference for preparing media for use as diluents or for gamete storage.

The correlation between the composition of the seminal plasma and the motility of spermatozoa has been investigated only in a few species: *Salmo salar* (Salmonidae) by Hwang and Idler (1969), *Cyprinus carpio* and *Alburnus alburnus* (Cyprinidae) by Kruger, Smith, VanVuren and Ferreira (1984) and Lahnsteiner *et al.* (1996), *Acipenser baeri* and *Acipenser fulvescens* by Gallis, Fedrigo, Jatteau, Bonpunt and Billard (1991) and Toth, Ciereszko, Christ and Dabrowski (1997). In this regard, compared with the physiology of reproduction of other fish, less attention has been paid to that of the male grass carp.

The objectives of the present study were (a) to determine the physical spermatological parameters and (b) to determine the major mineral and organic contents of the seminal plasma. An additional objective was to study the physiological relationships between the spermatological parameters and the mineral and organic contents of the seminal plasma.

Materials and methods

Collection of semen

The experiment was carried out at the State Hydrolic Works (SHW) Fish Reproduction Station, during the spawning season of grass carp. The male broodstock were held in sand ponds under a natural photoperiod regime and fasted 48 h before sperm collection. The males were anaesthetized in 100 ppm of MS 222 (Argent Labs., Redmond, WA, USA) before stripping. Sperm was collected from 25 mature males (TW = 3.27 ± 1.84 kg, TL = 45.23 ± 2.46 cm) by manual abdominal stripping 12 h after a single injection of 2 mg kg^{-1} of carp pituitary extract (CPE) at 22–24 °C water temperature. Sperm was sampled into glass tubes and used only if uncontaminated with water, blood, urine and faeces. The semen was stored in plastic plates separately for each fish and held on dry ice (4 °C) until measurement of sperm density within 1 day after stripping.

Evaluation of semen

An activating solution of 0.3% NaCl was used for estimating motility. For the evaluation of motility, about 10 μL of semen was placed on a glass microscope slide and 100 μL of activation solution was added. Motility was evaluated using a light microscope at ×200 magnification and was expressed as a percentage of motile spermatozoa. Each motility determination was performed in triplicate for each semen sample. The duration of sperm motility was estimated using a sensitive chronometer (1/100 s) until almost 90% of the sperm stopped their progressive movement. The spermatozoa density was estimated using the haemocytometric method.

With this aim, a droplet of the diluted milt was placed on a Thoma's haemocytometer slide (depth 0.1 mm) with a coverslip and counted using light microscopy. After a few minutes (to allow sperm sedimentation), the number of spermatozoa was counted at ×200 magnification and expressed as $\times 10^9$ mL^{-1}. Sperm pH was measured using standard pH electrodes within 30 min of sampling. Semen colour and consistency were evaluated visually and semen volume was measured in graduated tubes.

Determination of seminal plasma composition

Seminal plasma was collected after centrifugation of semen at 1370 *g* for 10 min at room temperature and stored in Eppendorf (Sigma-Aldrich, Taufkirchen, Germany) vials at −20 °C for 2 days until the beginning of the analyses. Seminal plasma was centrifuged twice to avoid possible contamination with spermatozoa. Major cations and metabolies such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, glucose, protein, cholesterol, triglyceride and urea levels were determined using an Abbott-Aeroset autoanalyser (Chicago, IL, USA) using original kits.

Statistical analysis

Data from individual fish on all other sperm quality parameters were analysed using one-way analysis of variance (ANOVA), followed by Duncan's new multiple

range test (DNMR) at a minimum significance of $P < 0.05$. Motility data were normalized through arcsine transformation. Correlations between physical spermatological parameters and seminal plasma composition were estimated using Pearson's correlation test. Results are presented as mean ± SEM. Statistical analyses were performed with SPSS 10 for Windows statistical software package.

Results

Spermatological parameters

Spermatological parameters of the collected sperm were found to be rather variable and are presented in Table 1. The sperm volume collected for each male ranged between 5 and 25 mL and the mean was found to be 14.44 ± 1.16 mL. Sperm volume showed a negative allometry with motility and movement duration ($P > 0.05$). The spermatozoa motility ranged between 70% and 95% and the mean was found to be 80.60 ± 1.55%. Spermatozoa motility and total spermatozoa density were negatively correlated ($r = -0.026$, $P > 0.05$). On the other hand, spermatozoa motility correlated positively with movement duration ($r = 0.513$, $P > 0.05$), density ($r = 0.021$, $P > 0.05$) and pH ($r = 0.246$, $P > 0.05$). Sperm was found to be viscous in consistency and creamy white in colour in all samples.

Seminal plasma composition

The compositions of the seminal plasma ion and metabolites are shown in Table 2. The ion content of the seminal plasma was found to be rather variable and high. The Na^+ and Ca^{2+} ions correlated negatively with spermatozoa motility ($r = -0.453$, $P > 0.05$ and $r = -0.192$, $P > 0.05$) respectively (Fig. 1a and c). Also, a negative allometry was found between spermatozoa motility and Mg^{2+} ($r = -0.401$, $P > 0.05$) (Fig. 1d). The K^+ ion correlated positively with spermatozoa motility ($r = 0.545$, $P > 0.05$) (Fig. 1b). A significant correlation was observed between sperm volume and triglyceride ($r = 0.736$, $P < 0.05$).

Table 1 Physical spermatological parameters of grass carp

	Minimum	Maximum	Mean	SEM
Volume (mL)	5	25	14.44	1.16
Motility (%)	70	95	80.60	1.55
Movement duration (s)	40	115	67.68	4.32
Density ($\times 10^9$ mL^{-1})	8.6	25.3	15.43	0.72
Total density ($\times 10^9$)	73	967.5	337.43	45.86
pH	6	8.5	7.24	0.17

Table 2 Seminal plasma ion and metabolite composition of grass carp

	Minimum	Maximum	Mean	SEM
Na^+ (mM L^{-1})	80	118	98.14	5.23
K^+ (mM L^{-1})	286	463	380.85	25.95
Ca^{2+} (mg dL^{-1})	4	47	30.25	4.96
Mg^{2+} (mEq L^{-1})	14	28	19.16	1.70
Glucose (mg dL^{-1})	0.7	1.8	1.36	0.11
Total protein (g dL^{-1})	0.1	0.8	0.37	0.08
Cholesterol (mg dL^{-1})	8	15	12.02	1.18
Triglyceride (mg dL^{-1})	7	19	14.85	1.50
Urea (mg dL^{-1})	11	91	43.5	9.56

The relationships between biochemical and spermatological properties are shown in Table 3. Spermatozoa motility correlated negatively with the metabolite composition of the seminal plasma ($P > 0.05$) (Fig. 1e–i). The Mg^{2+} ion correlated positively with glucose and cholesterol ($r = 0.692$, $P < 0.05$ and $r = 0.680$, $P < 0.05$) respectively. A highly significant positive relationship was also found between Mg^{2+} and total protein ($r = 0.837$, $P < 0.01$). On the other hand, a significantly negative relationship was detected between Ca^{2+} and triglyceride ($r = -0.639$, $P < 0.05$). Urea correlated significantly with glucose and triglyceride ($r = 0.640$, $P < 0.05$ and $r = 0.656$, $P < 0.05$) respectively. A highly significant relationship was also found between urea and cholesterol ($r = 0.857$, $P < 0.01$). Cholesterol also correlated significantly with total protein and triglyceride ($r = 0.702$, $P < 0.05$ and $r = 0.707$, $P < 0.05$) respectively.

Discussion

Sperm motility varies in vigour and duration not only among males but also within an individual male depending on ripeness (Tekin, Secer, Akcay, Bozkurt & Kayam 2003). Most studies on fish species have shown that the duration and motility of semen can vary seasonally (Benau & Terner 1980; Akcay, Bozkurt, Secer & Tekin 2004). The difference may be due to differences in feeding conditions, age, environmental factors, time of spawning or dilution ratio.

Knowledge of the physical and chemical constituents of spermatozoa and seminal plasma is a

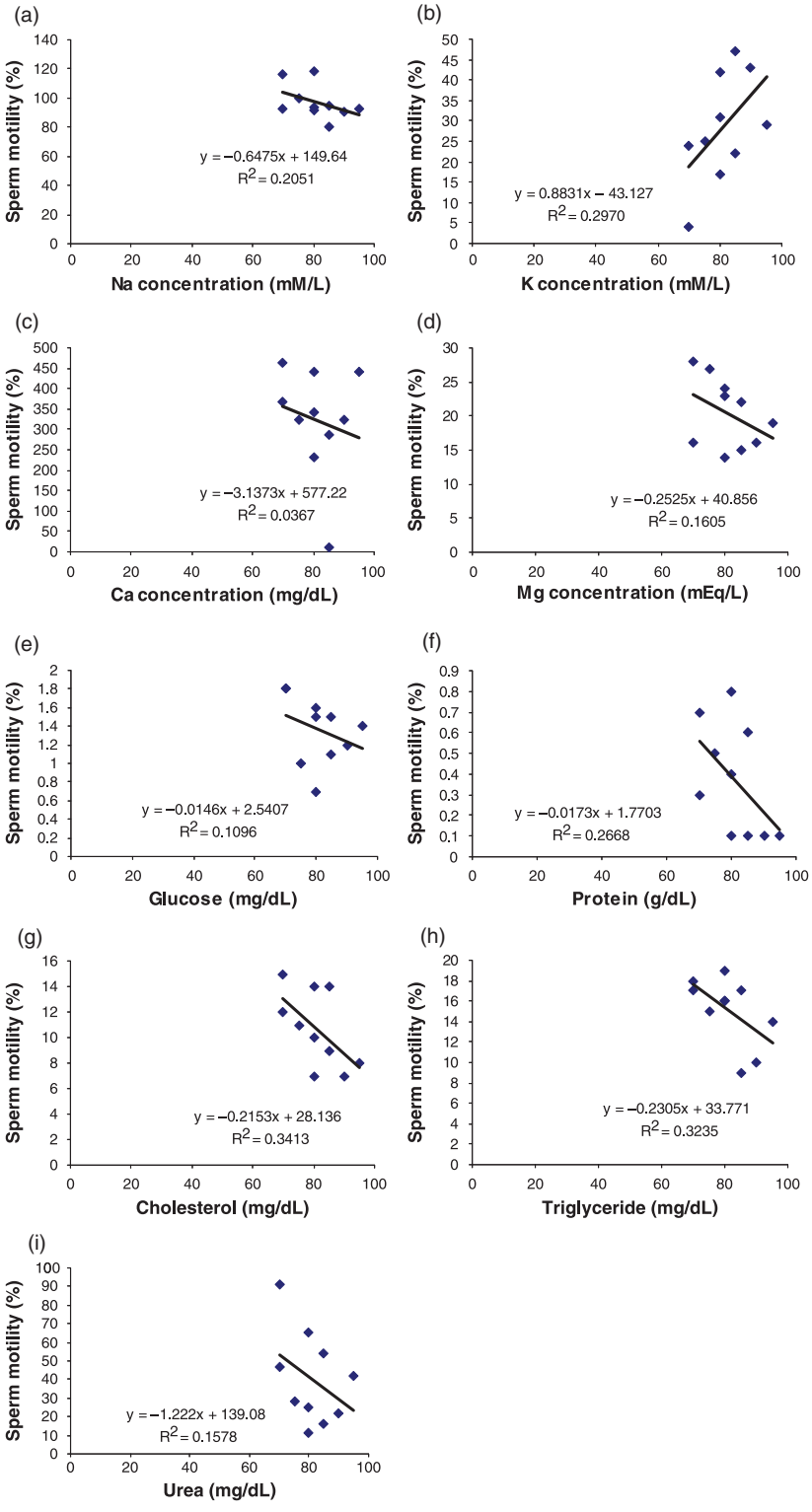

Figure 1 The relationships between sperm motility and (a) Na^+, (b) K^+, (c) Ca^{2+}, (d) Mg^{2+}, (e) glucose, (f) total protein, (g) cholesterol, (h) triglyceride, (i) urea. ($P > 0.05$, ANOVA).

Table 3 Linear correlations (*r*) between physical spermatological parameters and seminal plasma composition of grass carp sperm

	Volume	Motility	Movement duration	Density	Total density	pH	Na	K	Ca	Mg	Glucose	Total protein	Cholesterol	Triglyceride
Motility	−0.325													
Movement duration	−0.185	0.513												
Density	0.413	0.021	0.060											
Total density	0.535	−0.026	−0.002	0.959**										
pH	0.492	0.246	−0.258	0.167	0.293									
Na	−0.195	−0.453	−0.539	−0.195	−0.173	−0.289								
K	−0.200	0.545	0.140	−0.045	0.175	−0.017	0.189							
Ca	−0.141	−0.192	0.431	0.559	0.533	−0.020	−0.562	0.083						
Mg	0.071	−0.401	−0.264	−0.615	−0.547	−0.269	0.006	0.044	−0.408					
Glucose	0.226	−0.331	0.114	−0.435	−0.352	−0.289	−0.018	0.276	−0.176	0.692*				
Total protein	0.186	−0.517	−0.455	−0.417	−0.439	−0.307	0.239	−0.285	−0.603	0.837**	0.584			
Cholesterol	0.412	−0.584	−0.215	−0.409	−0.395	−0.063	−0.101	−0.067	−0.550	0.680*	0.428	0.702*		
Triglyceride	0.736*	−0.569	−0.542	−0.169	−0.038	0.418	0.105	0.102	−0.639*	0.535	0.325	0.604	0.707*	
Urea	0.535	−0.397	0.137	−0.384	−0.297	−0.030	−0.212	0.269	−0.446	0.614	0.640*	0.509	0.857**	0.656*

*Significant at $P<0.05$.
**Significant at $P<0.01$.

prerequisite for the successful evaluation of the reproductive ability of different fish species. Chemical criteria of importance are the presence or absence of inorganic and organic components in the semen. This may also lead to a better understanding of the mechanisms of fertilization.

Fish seminal plasma, in contrast to that of higher vertebrates, is characterized by a low total protein concentration, substantial mineral compounds [sodium (Na), potassium (K), calcium (Ca), magnesium (Mg)] and low concentrations of organic substances.

Seminal plasma of grass carp has a higher Na^+ content than common carp (75 mM L^{-1}; Morisawa, Suzuki, Shimizu, Morisawa & Yasuda 1983) and rainbow trout (80 mM L^{-1}; Secer, Tekin, Bozkurt, Bukan & Akcay 2004) but lower than perch (124 mmol L^{-1}; Lahnsteiner, Berger, Weismann & Patzner 1995), catfish (164 mmol L^{-1}; Tan-Fermin, Miura, Adachi & Yamauchi 1999) and muskellunge (129 mmol L^{-1}; Lin, Liu & Dabrowski 1996). However, the K^+ content of seminal plasma was higher than those reported for common carp (70 mmol L^{-1}; Morisawa *et al.* 1983), Atlantic salmon (28 mmol L^{-1}; Aas, Refstie & Gjerde 1991), perch (10 mmol L^{-1}; Lahnsteiner *et al.* 1995), catfish (18 mmol L^{-1}; Tan-Fermin *et al.* 1999) and muskellunge (28 mmol L^{-1}; Lin *et al.* 1996). These differences probably represent species-specific characteristics (Ciereszko *et al.* 2000).

The electrolytes ensure the viability of sperm. The K^+ ion has a specific role in maintaining spermatozoa in the quiescent state (Baynes, Scott & Dawson 1981). Low levels of the Na^+ and K^+ ions are associated with low percentages of motile spermatozoa and such semen should be considered to be of low quality. Also, low levels of Na^+ and K^+ may be caused by a deficiency in the formation of seminal plasma. K^+ has an inhibitory effect on the initiation of sperm motility in salmonids (Benau & Terner 1980; Billard & Cosson 1992; Lahnsteiner, Berger, Weismann & Patzner 1998), which is associated with a reduction in sperm-fertilizing ability (Kusa 1950), but this situation is not clear in cyprinidae. The percentage of motile cells of grass carp spermatozoa are observed to increase when the Ca^{2+} and Na^+ ion levels decrease and the K^+ ion level increases in the seminal plasma (Fig. 1a–c). The findings of this research indicated that K^+ increased the motility of grass carp sperm. This result was also supported by Billard and Cosson (1992), who obtained a similar finding with carp sperm. In contrast, it seems that Ca^{2+}, at certain concentrations tends to inhibit the spermatozoa.

The specific role of protein in fish semen is unknown. White and Macleod (1963) indicated that protein had a protective role. In this study, low concentrations of total protein (0.37 ± 0.08 g dL^{-1}) were found, which indicates a low demand for protein. The negative relationship of the protein with the K^+ and Ca^{2+} ions can be considered to be effective on sperm motility. However, because of the low correlation of protein with the sperm motility, the possible role of protein remains undefined. On the other hand, Lahnsteiner, Mansour and Berger (2004) found that seminal plasma proteins prolong the viability of rainbow trout spermatozoa as measured by sperm motility. Notable concentrations of urea (43.50 ± 9.56 mg dL^{-1}) were also found in the seminal plasma. Urea is considered in relationship with protein metabolism and total protein.

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 1.36 ± 0.11 mg dL^{-1}. The importance of glucose in fish semen is not clear. On the other hand, the presence of this sugar in seminal plasma has been connected to the high energy demand of the testes during spermatogenesis or for the lipid synthesis of spermatozoa (Soengas, Sanmartin, Barciela, Aldegunde & Rozas 1993).

Various lipid classes have been found in seminal plasma and their levels are highly variable among fish species, such as 0.007 g L^{-1} for Arctic charr and 1.00 g L^{-1} for Euroasian perch (Piironen & Hyvarinen 1983; Piironen 1994). In the present study, the mean level of triglyceride (14.85 ± 1.50 mg dL^{-1}) was negatively correlated with sperm motility. Triglycerides serve as energy resources for spermatozoa during immotile storage and during the regeneration phase after motility (Lahnsteiner, Patzner & Weismann 1993). According to Lahnsteiner, Patzner and Weismann (1994), low levels of triglycerides were found in the seminal plasma of cyprinids. Low triglyceride levels could therefore be indicative of inadequate energy resources, reduced motility rate and fertilization capacity.

Also, the cholesterol level was found to be 12.02 ± 1.18 mg dL^{-1} in this study. In spite of the identification of cholesterol in the seminal plasma of freshwater fish (Billard *et al.* 1995), there is not enough information about its role. Lipids and cholesterol probably might have a protective effect on environmental changes (especially on temperature) that might occur when fish semen are released.

The analysis of mineral and organic components of the seminal plasma revealed some species-specific characteristics, especially for K^+ and protein concentrations. In addition, several studies have indicated that the seminal plasma composition not only shows an inter-specific variation but also varies among different groups of fish of the same species (Linhart, Slechta & Slavik 1991; Alavi & Cosson 2006).

Conclusion

The present study on the quality of *Ctenoparyngodon idella* seminal plasma and its relationships with motility helps determine the optimal quality of sperm to be used for artificial fertilization purposes. It also provides a better knowledge of the changes of the ionic content of seminal plasma as well as intracellular ionic content, which could be very useful to improve cryopreservation techniques used for long-term conservation of fish sperm.

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