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Assessment of Reproductive Parameters in Silver Carp (*Hypophthalmichthys molitrix*)

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Abstract: The present study was conducted to evaluate seminal plasma composition, spermatological parameters and their relationships in silver carp (*Hypophthalmichthys molitrix*). Eleven mature silver carp males (TW = 2.45±1.24 kg, TL = 46.23±2.52 cm) were randomly selected from the stock and were used as semen donors in the middle of the spawning season. For sperm collection, fish were not fed 48 h prior to collection and the males were anaesthetized in 5 mg L^{-1} of quinaldine sulphate before stripping. Before collection of sperm, the genital area was cleaned with a towel and dried to avoid contamination of sperm with feces, urine and water. Then, a gentle abdominal pressure was applied 12 h after a single injection of 2 mg g^{-1} body weight of Carp Pituitary Extract (CPE) at 23-24°C water temperature. Sperm was sampled into the calibrated glass tubes and the volume was expressed as mL. Sperm motility was estimated from freshly collected samples of milt according to the percentage of motile spermatozoa. Collected sperm was immediately transported to the laboratory for analyses. Seminal plasma contained 71.18±3.40 mM L-1 (Na^+), 87.48±4.42 mM L-1 (K^+), 10.64±1.50 mg dL-1 (Ca^{2+}), 2.57±0.19 mEq L-1 (Mg^{2+}), 0.12±0.04 g dL-1 total protein, 1.43±0.02 mg dL-1 glucose, 7.82±2.60 mg dL-1 triglyceride, 6.27±2.32 mg dL-1 cholesterol, 24.45±7.98 mg dL-1 urea. The following spermatological parameters were recorded: sperm volume 13.17±5.39 mL, sperm motility 63.18±7.16%, movement duration 56.81±20.32 s, sperm concentration 29.637±1.07x109 mL-1, total sperm concentration 419.200±29.62x109 and pH 7.59±0.74. The K^+ and Ca^{2+} ions correlated positively with motility ($r = 0.215$, $p>0.05$ and $r = 0.048$, $p>0.05$), respectively. On the other hand, Na^+ and Mg^{2+} ions correlated negatively with motility ($r = -0.067$, $p>0.05$, $r = -0.407$, $p>0.05$), respectively. Significant correlations were observed between K^+ and Na^+ ($r = -0.695$, $p<0.05$), protein and triglyceride ($r = 0.786$, $p<0.01$), protein and cholesterol ($r = 0.936$, $p<0.01$), triglyceride and cholesterol ($r = 0.802$, $p<0.01$). These parameters can be used to prepare species-specific extenders for short-term storage and cryopreservation of silver carp sperm.

Key words: Seminal Plasma % Silver Carp % Spermatological

INTRODUCTION

Broodfish management is an important factor that influence gamete quality and success of artificial insemination [1]. Better knowledge of sperm biochemistry, physiology and the mechanisms of reproduction are essential to improve artificial fertilization procedures for fish.

Evaluation of sperm quality provides information allowing aquaculturists to determine the optimal time for sperm collection and to devise optimal handling and storage protocols for sperm used in artificial fertilization [2]. Sperm volume, concentration and motility, as well as composition of the seminal plasma are common parameters to assess sperm quality in fish [3,4].

The composition of seminal plasma has great influence on the biological quality of the milt, as expressed by sperm viability and motility. These factors are directly related to the fertilization success. Determining of seminal plasma composition can help to understand the design requirements for preparing the appropriate artificial seminal plasma solutions for short-term storage or cryopreservation.

The inorganic compounds provide isotonic conditions for spermatozoa and especially, K^+ ions prevent sperm motility in some freshwater fish species such as salmonids and sturgeons [5]. Also, some organic compounds including glucose and triglyceride act as the main energy resources for spermatozoa metabolism [6] and some others such as proteins prolong the viability of

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spermatozoa [7]. To increase the efficiency of artificial fertilization, the composition of sperm diluents is very important and must be according to the species-specific composition of the seminal plasma.

Compared to the physiology of reproduction of other fishes, less attention has been paid to that of the silver carp. Based on the literature, sperm characteristics of silver carp has been rarely investigated. So, this study was designed to determine the physical and biochemical characteristics of silver carp sperm and to investigate their physiological relationships with each other.

MATERIALS AND METHODS

Broodstock Care and Collection of Semen: The experiment was carried out at the Fish Reproduction Station. The male broodstock were held in sand ponds (water temperature: 23-24°C, O_2: 7-8 ppm) under a natural photoperiod regime and fasted 48 h before semen collection. Eleven mature silver carp males (TW = 2.45±1.24 kg, TL = 46.23±2.52 cm) were randomly selected from the stock and were used as semen donors in the middle of the spawning season. For sperm collection, fish were not fed 48 h prior to collection and the males were anaesthetized in 5 mg L^{-1} of quinaldine sulphate before stripping. Before collection of sperm, the genital area was cleaned with a towel and dried to avoid contamination of sperm with feces, urine and water. Then, a gentle abdominal pressure was applied 12 h after a single injection of 2 mg g^{-1} body weight of Carp Pituitary Extract (CPE) at 23-24°C water temperature. Collected sperm was immediately transported to the laboratory for analyses.

Evaluation of Semen: Sperm was sampled into the calibrated glass tubes and the volume was expressed as mL. Sperm motility was estimated from freshly collected samples of milt according to the percentage of motile spermatozoa. For this aim, a 5 µL drop of sperm was placed directly on a microscope slide and 100 µL of activation solution (0.3% NaCl) was added. Microscopic observation at 400x magnification was carried out at room temperature (20°C). Three fields of view were examined for each slide and three aliquots of each milt sample were inspected for calculation of an average. Duration of spermatozoa movement was assessed using a sensitive chronometer (1/100) that was started simultaneously with the addition of activation solution into the samples.

Spermatozoa concentration was determined using the haemacytometric method. For this aim, sperm was diluted at ratio of 1:1000 with Hayem solution (5 g Na_2SO_4, 1 g NaCl, 0.5g $HgCl_2$ 200 mL bidistilled water). Ten microliter of the dilution were taken for counting on a Thoma hemocytometer slide (depth 0.1 mm). To prevent the spermatozoa adhering to the slides, they were previously bathed in a 10% bovine serum albumin solution (30 min, 4°C), then washed with distilled water and dried in a hood. Mean spermatozoa count was calculated from three replicate samples for each fish at magnification of 400x and expressed as $x10^9$ mL^{-1}. Sperm pH was measured using standard pH papers (Merck) within 30 min of sampling. Semen colour was evaluated visually immediately following sperm collection.

Determination of Seminal Plasma Composition: Seminal plasma was collected after centrifugation of semen at 4000x g for 10 min at room temperature (20°C) and stored in Eppendorf (Wiesbaden, Germany) vials at -20°C, until the beginning of the analyses. Seminal plasma was centrifuged twice to avoid possible contamination with spermatozoa. Major cations and metabolites such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, glucose, protein, cholesterol, triglyceride and urea levels were determined using an Abbott-Aeroset autoanalyser (Chicago, USA) using original kits.

Statistical Analisis: 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 7.4 and 23.4 mL and the mean was found to be 13.17±5.39 mL. Sperm volume correlated significantly with motility ($r = 0.653$, $p<0.05$) and total concentration ($r = 0.834$, $p<0.01$). The spermatozoa motility ranged between 60 and 75% and the mean was found to be 63.18±7.16. Sperm was found to be viscous in consistency and creamy white in colour in all samples.

Table 1: Spermatological parameters of silver carp (n = 11)

Parameters	Minimum	Maximum	Mean	SEM
Volume (mL)	7.400	23.400	13.170	5.39
Motility (%)	60.000	75.000	63.180	7.16
Movement duration (s)	28.000	85.000	56.810	20.32
Density (×109 mL-1)	20.270	52.750	29.637	1.07
Total density (×109)	200.100	967.200	419.200	29.62
pH	6.900	9.200	7.590	0.74

Table 2: Seminal plasma ion and metabolite composition of silver carp sperm (n = 3)

Variables	Minimum	Maximum	Mean	SEM
Na+ (mM L^{-1})	68.1	77.4	71.18	3.40
K+ (mM L^{-1})	81.4	97.1	87.48	4.42
Ca2+ (mg dL^{-1})	7.5	13.3	10.64	1.50
Mg2+ (mEq L^{-1})	2.3	2.9	2.57	0.19
Glucose (mg dL^{-1})	1.2	1.8	1.43	0.02
Total Protein (g dL^{-1})	0.1	0.4	0.12	0.04
Triglyceride (mg dL^{-1})	5.0	12.0	7.82	2.60
Cholesterol (mg dL^{-1})	5.0	11.0	6.27	2.32
Urea (mg dL^{-1})	10.0	37.0	24.45	7.98

Table 3: Correlations between spermatological parameters and seminal plasma composition of silver carp sperm

Variables	Volume	Motility	Movement duration	Density	Total density	pH	Ca	Na	K	Mg	Glucose	Protein	Triglyceride	Cholesterol
Motility	0.653*													
Movement duration	-0.444	0.542												
Density	0.549	0.417	-0.293											
Total density	0.834**	0.602	-0.408	0.914**										
pH	-0.219	-0.013	0.359	0.049	-0.123									
Ca	-0.105	0.048	0.277	-0.199	-0.152	0.054								
Na	0.330	-0.067	0.145	-0.282	-0.043	-0.127	-0.249							
K	-0.256	0.215	-0.559	-0.149	-0.209	-0.112	0.147	-0.695*						
Mg	0.013	-0.407	0.198	-0.496	-0.304	-0.417	0.213	0.271	-0.164					
Glucose	0.165	0.376	-0.199	-0.069	0.052	-0.040	0.396	0.061	0.069	0.137				
Protein	-0.001	-0.285	-0.353	-0.274	-0.202	0.008	-0.015	0.155	0.129	0.430	0.261			
Triglyceride	-0.236	-0.234	-0.467	-0.360	-0.361	-0.079	-0.024	0.027	0.418	0.029	0.141	0.786**		
Cholesterol	-0.269	-0.537	-0.155	-0.350	-0.379	0.060	-0.089	0.107	0.072	0.380	0.052	0.936**	0.802**	
Urea	0.183	-0.028	-0.447	-0.157	-0.047	-0.001	-0.545	0.162	0.186	0.273	-0.343	0.527	0.318	0.472

*Significant at p<0.05, **Significant at p<0.01

Seminal Plasma Composition: The seminal plasma ionic and metabolite composition are shown in table 2. Main ionic components such as Na^+, K^+, Ca^{2+} and Mg^{2+} were found rather variable. There were no significant difference among Ca^{++}, K^+ and motility (r = 0.048, r = 0.215) respectively. On the other hand, negative relationship was determined between motility, Na^+ and Mg^{2+} ions (r = -0.067, p>0.05; r = -0.407, p>0.05) (Table 3).

Spermatozoa motility showed negative relationship with the metabolites of the seminal plasma such as protein, triglyceride, cholesterol and urea (r = -0.285, p>0.05; r = -0.234, p>0.05; r = -0.537, p>0.05; r = -0.028, p>0.05), but positive relationship was determined with glucose (r = 0.376, p>0.05), respectively. On the other hand, spermatozoa concentration showed negative allometry with all ionic and metabolic composition of the seminal plasma (p>0.05). Protein significantly correlated with triglyceride and cholesterol (r = 0.786, p<0.01; r = 0.936, p<0.01). A highly significant relationship was also found between triglyceride and cholesterol (r = 0.802, p<0.01).

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DISCUSSION

Mean semen volume was similar with the findings of Akcay *et al*. [8] for mirror carp, but differed from results reported by Bozkurt and Secer [9] for mirror carp. The differences may be due to the feeding conditions and regime, water quality, environmental factors or spawning time. In the present study, mean sperm motility and motility duration was determined as 63.18±7.16% and 56.81±20.32 sec, respectively. Spermatozoa motility varies in vigor and duration not only among males but also within an individual male depending on ripeness [10] and probably due to contamination with urine during

stripping. Also, most studies on fish species have shown that the duration and motility of semen can vary seasonally [8, 11]. Spermatozoa concentration showed great differences with the findings of Emri *et al*. [12], Akcay *et al*. [8] and Bozkurt and Secer [9]. The differences may be due to differences in feeding conditions, age, environmental factors, time of spawning, or dilution ratio.

According to the results of the present study, seminal plasma of silver carp has lower Na^+ content (71.18±3.40 mM L^{-1}) than other freshwater species such as grass carp (98 mmol L^{-1}) [13], rainbow trout (80 mmol L^{-1}) [14], perch (124 mmol L^{-1}) [15]. On the other hand, the concentration of K^+ (87.48±4.42 mM L^{-1}) in the seminal plasma was higher than in Atlantic salmon ((28 mM L^{-1}) [16], *Salmo trutta abanticus* (38 mM L^{-1}) [17], rainbow trout ((46 mM L^{-1}) [14] and perch (10 mM L^{-1}) [15].

The electrolytes ensure the viability of sperm. Low level of the Na^+ ion can be associated with low percentages of motility and may be caused by a deformation of seminal plasma. The K^+ ion has a specific role in maintaining spermatozoa in the quiescent state [18]. K^+ has an inhibitory effect on the initiation of sperm motility in salmonids [19]. Similarly, findings of the present study agree with the inhibitory effect of K^+ ion on motility like in salmonids. The percentage of motile cells of silver carp spermatozoa are observed tend to increase when, the Mg^{2+} ion level in decrease. It seems that Mg^{2+} at certain concentrations tends to inhibit the spermatozoa in silver carp.

The specific role of protein in fish semen is unknown. White and Macleod [20] indicated that protein had a protective role. In this study, low concentrations of total protein (0.12± 0.04 g dL^{-1}) were found, which indicates a low demand for protein. However, because of the negative relationship of protein with the sperm motility the possible role of protein remains underfined. On the other hand, Lahnsteiner *et al*. [7] found that seminal plasma proteins prolong the viability of rainbow trout spermatozoa as measured by sperm motility. Notable concentrations of urea (24.45±7.98 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 1.43±0.02 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 [21].

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 [22]. In the present study, the mean level of triglyceride (7.82±2.60 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 [6]. According to Lahnsteiner *et al*. [23], 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 6.27±2.32 mg dL^{-1} in this study. In spite of the identification of cholesterol in the seminal plasma of freshwater fish [1], 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.

CONCLUSIONS

The results of this study confirmed differences in the compositions of seminal plasma in related species. The greatest changes in the ionic composition of the seminal plasma in silver carp has been noted in the K^+ ion concentrations. This study also, suggests that the concentration of the organic and inorganic compounds in artificial seminal plasma should be modified to meet species specific extender requirements for short-term storage or cryopreservation of silver carp sperm.

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