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ISSN 0792 - 156X

PUBLISHER:
Israeli Journal of Aquaculture - BAMIGDEH -
Kibbutz Ein Hamifratz, Mobile Post 25210,
ISRAEL
Phone: + 972 52 3965809
http://siamb.org.il

Effects of Seminal Plasma Composition on Sperm Motility in Mirror Carp (*Cyprinus carpio*)

Yusuf Bozkurt*[1], Fatih Ogretmen[2], Faik Sertel Secer[3], Ugur Ercin[4]

[1] *Department of Aquaculture, Faculty of Fisheries, Mustafa Kemal University, Hatay, Turkey*

[2] *State Hydraulic Works, Fish Production Station, Adana, Turkey*

[3] *Department of Aquaculture, Faculty of Agriculture, Ankara University, Ankara, Turkey*

[4] *Department of Biochemistry, Faculty of Medicine, Gazi University, Ankara, Turkey*

(Received 31.3.09, Accepted 7.04.09)

Key words: seminal plasma, motility, sperm quality, mirror carp

Abstract

This research provides data concerning the biochemical (ionic and organic) composition of sperm of the mirror carp (*Cyprinus carpio*) and its relationship to spermatozoa motility. Seminal plasma contained 67.12±1.06 mM/l Na^+, 105.1±2.24 mM/l K^+, 7.85±0.67 mg/dl Ca^{2+}, 2.61±0.11 mEq/l Mg^{2+}, 0.14±0.002 g/dl total protein, 10.3±1.01 mg/dl triglyceride, 6.83±0.72 mg/dl cholesterol, and 54.72±3.49 mg/dl urea. A positive relationship ($p<0.05$) was determined between Na^+ and motility ($r = 0.522$). On the other hand, Ca^{2+}, K^+, and Mg^{2+} ions negatively correlated ($p>0.05$) with motility ($r = -0.565$, $r = -0.160$, and $r = -0.184$, respectively). Spermatozoa motility correlated negatively ($p>0.05$) with protein ($r = -0.233$), triglyceride ($r = -0.348$), and urea ($r = -0.331$) but positively with cholesterol ($r = 0.012$). This information will help to develop cryopreservation procedures, to meet species-specific extender requirements, and to optimize artificial fertilization procedures in mirror carp.

Introduction

Gamete management is key to achieving high fertilization success in fish farms (Billard et al. 1995). Studies on sperm physiology can provide basic knowledge for managing fertilization (Alavi and Cosson, 2005). For controlled and successful production in aquaculture systems, it is necessary to have adequate knowledge of the physical and chemical characteristics of the milt to determine the reproductive ability of cultivated fish. The ionic and organic constituents of the seminal fluid can indicate fish fertilization capacity (Ciereszko et al., 2000).

* Corresponding author. E-mail: yfbozkurt@yahoo.com

Seminal plasma has a unique composition. Organic and inorganic electrolytes support the viability of spermatozoa cells. Other substances reflect the functioning of the whole reproductive system including sperm cells (Piironen and Hyvarinen, 1983; Ciereszko et al., 2000). The composition of seminal plasma influences the biological quality of the milt, as expressed by sperm viability and motility. These factors are directly related to fertilization success (Aas et al., 1991). Sperm motility is an important indicator of sperm quality and is usually expressed as the percentage and duration of motile sperm after activation. Studies on semen characteristics help to understand the basic biochemical processes that occur in sperm motility (Ingermann et al., 2002; Kowalski et al., 2003) and evaluate the reproductive ability of fish. Thus, basic knowledge of the chemical composition of seminal plasma and sperm motility are important for determining the composition of sperm-activating or immobilizing solutions.

As far as we know, the relationship between the seminal plasma composition and sperm motility in mirror carp (*Cyprinus carpio*) has rarely been investigated (Kruger et al., 1984; Gallis et al., 1991; Lahnsteiner et al., 1996). The aim of the present study was to examine the major mineral and organic contents of seminal fluid of mirror carp and determine the relationship between the biochemical contents and spermatozoa motility.

Materials and Methods

Broodstock care and sperm collection. Male broodstock were held in sand ponds under a natural photoperiod regime. Eighteen mature mirror carp males (total wt 2.73±0.54 kg, total length 42.61±1.27 cm) were randomly selected from the stock for use as semen donors. The fish were not fed 48 h prior to sperm collection. Fish were given a single injection of 2 mg/kg of carp pituitary extract (CPE) at 22-24°C water temperature. Fish were anesthetized in 5 mg/l of Quinaldine and sperm was collected 12 h after injection by manual abdominal stripping. Sperm was used only if uncontaminated by water, blood, urine, or feces and was immediately transported on ice (4°C) to the laboratory for analyses.

Spermatozoa motility. Sperm motility was estimated in freshly collected samples of milt. For this aim, a 10 µl drop of sperm was placed on a microscope slide and 100 µl of activation solution (0.3% NaCl) was added. The percent of motile spermatozoa was determined visually by one person at x200 magnification at room temperature (20°C). Three fields of view were examined for each slide, and three aliquots of each milt sample were inspected to calculate an average. The duration of spermatozoa movement was assessed using a sensitive chronometer (1/100) that was started simultaneously with the addition of the activation solution to the sample.

Sperm volume, density, pH, and color. Sperm was sampled into 25-ml calibrated glass cylinders and the volume was expressed as ml. Spermatozoa density was determined according to the hemacytometric method, i.e., the sperm was diluted at a ratio of 1:1000 with Hayem solution (5 g Na_2SO_4, 1 g NaCl, 0.5 g $HgCl_2$, and 200 ml bidistilled water) and the mean spermatozoa count was calculated from three replicate samples for each fish at a magnification of x200. Spermatozoa density was expressed as $x10^9$/ml. Counting chambers were kept in a moist atmosphere for at least 10 min before cell counting. Sperm pH was measured using standard pH papers (Merck) within 30 min of sampling. Semen color was evaluated visually immediately following collection.

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

Statistical analysis. Sperm quality parameters from individual fish were analyzed using one-way analysis of variance (ANOVA), followed by Duncan's new multiple range test (DNMR) with 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 means±SEM. Statistical analyses were performed with the SPSS 10 for Windows statistical software package.

Results

Sperm was viscous and creamy white in all samples. Spermatological properties are presented in Table 1. Ionic and organic composition is given in Table 2. Glucose and Cl^- were not detected. Semen volumes, Na^+, K^+, Ca^{2+}, and Mg^{2+} were rather variable. Correlations between the biochemical and spermatological properties are presented in Table 3. Motility correlated negatively with Ca^{2+} and positively with volume and Na^+ (Fig. 1). Total density correlated positively with volume and Mg^{2+} but negatively with urea. Protein correlated positively with Ca^{2+} and triglyceride.

Discussion

In all animal species, sperm quality data are required to determine successful artificial insemination and semen handling techniques. Motility is the most commonly used parameter to evaluate sperm quality in fishes (Billard et al., 1995; Lahnsteiner and Patzner, 1998). In general, sperm must be motile to achieve fertilization, and low fertility rates are correlated with sperm samples that contain a low percentage of motile sperm.

Table 1. Spermatological parameters of mirror carp (n = 18).

	Minimum	*Maximum*	*Mean*	*SEM*
Volume (ml)	3.8	18.2	9.09	0.87
Motility (%)	54	91	75	2.35
Movement duration (s)	27	52	40.49	1.43
Density (x 10^9/ml)	18,250	39,000	28,795	1247.17
Total density (x 10^9)	117,420	416,000	254,339	19,525.4
pH	5	9	7.15	0.23

Table 2. Seminal plasma ion and metabolite composition* of mirror carp sperm (n = 3).

	Minimum	*Maximum*	*Mean*	*SEM*
Na^+ (mM/l)	57	76	67.12	1.06
K^+ (mM/l)	92.1	130.5	105.1	2.24
Ca^{2+} (mg/dl)	3.7	11.8	7.85	0.67
Mg^{2+} (mEq/l)	1.5	3.3	2.61	0.11
Total protein (g/dl)	0.1	0.2	0.14	0.002
Triglyceride (mg/dl)	4	20	10.3	1.01
Cholesterol (mg/dl)	4	14	6.83	0.72
Urea (mg/dl)	38	97	54.72	3.49

* Glucose and Cl^- were not detected.

Table 3. Correlations between spermatological parameters and seminal plasma composition of mirror carp sperm.

	Volume	*Motility*	*Movement duration*	*Density*	*Total density*	*pH*	*Ca*	*Na*	*K*	*Mg*	*Protein*	*Triglyceride*	*Choles-terol*
Motility	0.479*	-	-	-	-	-	-	-	-	-	-	-	-
Movement duration	0.161	0.100	-	-	-	-	-	-	-	-	-	-	-
Density	-0.414	-0.184	-0.210	-	-	-	-	-	-	-	-	-	-
Total density	0.843**	0.432	0.082	0.120	-	-	-	-	-	-	-	-	-
pH	0.412	0.433	0.453	-0.253	0.337	-	-	-	-	-	-	-	-
Ca	-0.261	-0.565*	0.398	0.109	-0.193	-0.284	-	-	-	-	-	-	-
Na	0.316	0.522*	-0.188	-0.037	0.378	-0.137	-0.241	-	-	-	-	-	-
K	0.121	-0.160	-0.284	0.083	0.159	0.008	0.115	0.039	-	-	-	-	-
Mg	0.441	-0.184	0.316	-0.043	0.495*	0.070	0.344	-0.129	-0.108	-	-	-	-
Protein	0.007	-0.233	0.071	-0.059	0.009	-0.176	0.590**	0.090	0.296	0.349	-	-	-
Triglyceride	-0.014	-0.348	-0.098	-0.028	-0.019	-0.337	0.318	0.176	-0.087	0.158	0.556*	-	-
Cholesterol	0.021	0.012	-0.173	-0.022	0.016	-0.206	0.019	0.339	-0.216	-0.031	0.352	0.829**	-
Urea	-0.414	-0.331	-0.036	-0.098	-0.531*	-0.408	0.551	-0.067	0.334	-0.325	0.333	0.238	0.126

*Significant at $p<0.05$
**Significant at $p<0.01$

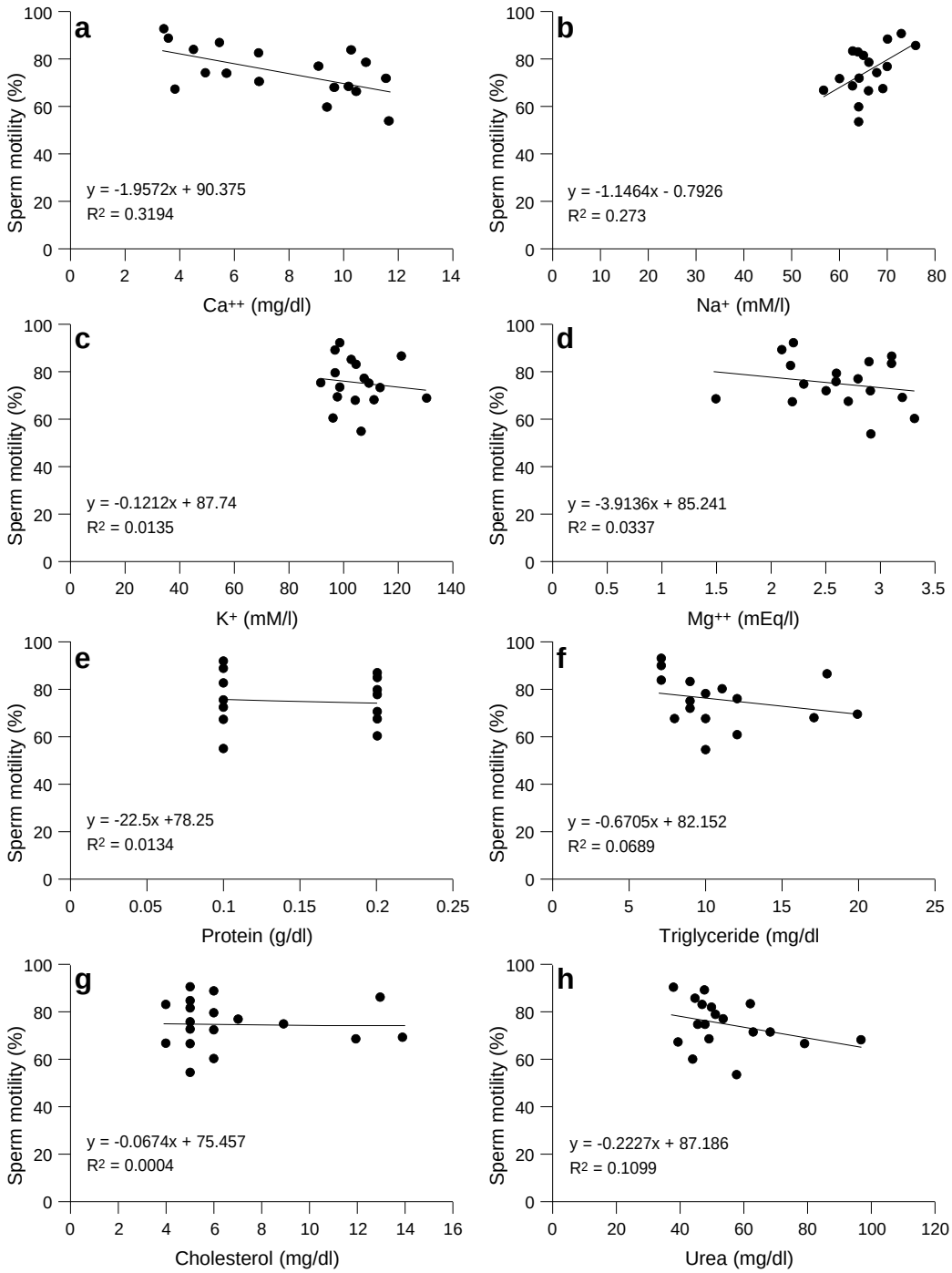

Fig. 1. The relationships between sperm motility and (a) Ca^{2+}, (b) Na^{+}, (c) K^{+}, (d) Mg^{2+}, (e) protein, (f) triglyceride, (g) cholesterol, and (h) urea (p<0.05, ANOVA).

In the present study, mean sperm motility was 75±2.35%. Motility varies in vigor and duration not only among males but also within an individual male depending on ripeness (Tekin et al., 2003). Most studies on fish species have shown that the duration and motility of semen can vary seasonally (Benau and Terner, 1980; Akcay et al., 2004). The difference may be due to differences in feeding conditions, age, environmental factors, time of spawning, dilution ratio, and ionic composition of the seminal plasma.

The ionic composition of seminal plasma has an important influence on sperm motility in fish that fertilize externally. For example, a high K^+ concentration inhibits sperm motility in salmonids (Morisawa and Suzuki, 1980) but increases sperm motility in carp (Billard and Cosson, 1992). In our study, the K^+ concentration (105.1±2.24 mM/l) was higher than in Atlantic salmon (28 mM/l; Aas et al., 1991), *Salmo trutta abanticus* (38 mM/l; Bozkurt, 2008), rainbow trout (46 mM/l; Secer et al., 2004), perch (10 mM/l; Lahnsteiner et al., 1995), and Asian catfish (18 mmol/l; Tan-Fermin et al., 1999). In spite of the higher K^+ concentration, motility values were lower in our study than in the studies mentioned above.

Seminal plasma of mirror carp has a lower Na^+ content (67.12±1.06 mM/l) than other freshwater species such as grass carp (98 mmol/l; Bozkurt et al., 2008), rainbow trout (80 mmol/l; Secer et al., 2004), perch (124 mmol/l; Lahnsteiner et al., 1995), Asian catfish (164 mmol/l; Tan-Fermin et al., 1999), and muskellunge (129 mmol/l; Lin et al., 1996). The negative relationship between K^+ and motility in the present study shows that motility in mirror carp sperm depends more on the Na^+ ion concentration than on the K^+ iion concentration. The low Na^+ level may be due to a deficiency in the formation of seminal plasma and consequently can be associated with the low percentage of motile spermatozoa. Hence, semen with a low Na^+ concentration should be considered low quality.

Ca^{2+} and Mg^{2+} also contribute significantly to the ionic composition of seminal plasma in fish milt. Divalent cations (mainly Ca^{2+} and Mg^{2+}) are more effective in antagonizing the inhibitory effect of K^+ on sperm motility than the monovalent Na^+ ion (Baynes et al., 1981; Billard and Cosson, 1992).

The present research shows that a low concentration of total protein (0.14±0.002 g/dl) does not affect sperm motility. This may indicate a low demand for protein in mirror carp seminal plasma in contrast to findings that seminal plasma proteins prolong the viability of rainbow trout, as measured by sperm motility (Lahnsteiner et al., 2004). Notable concentrations of urea (54.72±3.49 mg/dl) were found in the seminal plasma. Urea is considered in relationship with protein metabolism and total protein because it occurs as a result of the digestion of protein, which contains N_2.

Lipid levels are highly variable among fish species, e.g., 0.007 g/l for Arctic charr and 1.00 g/l for Euroasian perch (Piironen and Hyvarinen, 1983; Piironen, 1994). In the present study, the mean triglyceride level (10.3±1.01 mg/dl) was negatively correlated with sperm motility. Triglycerides serve as an energy resource for spermatozoa during immotile storage and the regeneration phase after motility (Lahnsteiner et al., 1993). Our results agree with Lahnsteiner et al. (1994) that the triglyceride level in the seminal plasma of cyprinids is low. A low triglyceride level can therefore indicate inadequate energy resources, a reduced motility rate, and reduced fertilization capacity.

In this study the cholesterol level was 6.83±0.72 mg/dl. 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 might have a protective effect against environmental changes (especially in temperature) that occur when fish semen is released (Bozkurt et al., 2008).

We conclude that the main reason for the low motility was a lack of glucose in the seminal plasma. A rapid decrease in motility after activation is related to diminution of the intracellular ATP which depends on glucose. Piironen and Hyvarinen (1983) noted a zero glucose concen-

tration and low motility in *S. trutta m. lacustris*. The lack of glucose in the semen can be explained by the spawning stage or contamination with bacteria that quickly decomposes glucose in sperm during transportation.

The results of the present study indicate that the ionic composition of seminal plasma is an important factor determining motility in mirror carp. The mineral and organic components revealed species-specific characteristics such as low triglyceride. The correlation between ionic composition and sperm quality provides a good indication of the composition to be used in extenders for mirror carp when optimal cryopreservation procedures are formulated.

Acknowledgements

The authors are grateful to the staff of the SHW Fish Production Station in Adana, Turkey. The authors also would like to thank Dr. N. Bukan for providing necessary materials and conditions for analysis of the seminal plasma.

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