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EFFECT OF pH AND IONS ON MOTILITY OF SCALY CARP (*Cyprinus carpio*) SPERMATOZOA

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Abstract. This study investigated the effects of environmental factors including pH and cations such as sodium (Na^+), potassium (K^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}) concentrations on sperm motility of scaly carp (*Cyprinus carpio*). Sperm motility was evaluated by the duration of sperm movement and the initial percentage of motile sperm. The maximum percentage of motile sperm and total duration of sperm motility were observed in solutions containing 25 mM NaCl (95.0±16.0% and 147.0±5.27 s), 0.5 mM KCl (85.0±2.45% and 174±2.47 s), 5 mM $CaSO_4$ (90.0±2.27% and 95.2±3.46 s) and 10 mM $MgSO_4$ (80.16±2.45% and 124.56±26.42 s). In addition, maximum and minimum percentages and total durations of motility occurred at pH 8.0 and pH 6.0, respectively. The prolonged duration of movement might be caused by reactivation of sperm or gradual activation of sperm motility. Concentrations more than 50 mM of Na^+, 2 mM of K^+, 10 mM of Ca^{2+}, 10 mM of Mg^{2+} and more than pH 8 had negative effects on sperm motility. The present study provides us with some basic knowledge about scaly carp spermatozoa biosensitivity to the ionic media.

Keywords: motility, sperm quality, ions, pH, scaly carp.

AIMS AND BACKGROUND

During natural reproduction, fish spermatozoa become motile following discharge into the aqueous environment[1]. Fish spermatozoa are important since they are released usually in a hostile external medium where they have to cope with extremely harmful conditions.

In the environment encountered by spermatozoa following release into the natural conditions or after dilution in artificial fertilisation media, definite ion concentrations (Na^+, K^+, Ca^{2+}, Mg^{2+}, etc.), osmolality and pH are crucial parameters. As these conditions depolarise the cell membrane, they may affect the capacity of sperm tails for flagellar motility and may stimulate this motility[2]. Motility duration which lasts for short periods, initiation of motility and motility patterns are

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especially well documented for rainbow trout (*Oncorhynchus mykiss*) sperm but very little information presently deals with sperm motility characteristics in other fish species[3].

The activation of sperm motility occurs in response to the changes in the external medium. Among the external factors, particularly ions and pH play a vital role in sperm physiology in fish. The quality of sperm is a major factor contributing to the successful production of fish larvae. Fish spermatozoa are generally characterised by short-lasting motility. Considering the short time for fertilisation following releasing of sperm into the aquatic environment, any negative effect on sperm motility may dramatically decrease fertilisation success[4]. It is known that sperm motility is sensitive and reliable indicator of aquatic pollution[5].

To increase the efficiency of artificial fertilisation, the biochemical composition of sperm diluents is very important and must be adjusted according to the species specific composition of the seminal plasma[6]. Also pH of the activating solution usually affects motility, but in a low extent. It was reported that alkaline pH of the activation solution gives better motility in rainbow trout spermatozoa[7]. On the other hand, few observations are reported on the effects of the environmental conditions for scaly carp (*Cyprinus carpio*) spermatozoa motility. It is necessary to pay attention to the other factors such as diluent pH and presence of ionic pollutants. For this reason, the aim of the present study was to determine the effect of different ions and pH on motility.

EXPERIMENTAL

Broodstock care and sperm collection. The experiment was carried out at the State Hydraulic Works (SHW) Fish Reproduction Station, Adana, Turkey, during the spawning season of scaly carp. The male broodstock were held in sand ponds under a natural photoperiod regime. 15 mature scaly carp males (total weight 2.27±0.72 kg, total length 43.27±2.17 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 anaesthetised 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.

Determination of sperm motility. An activating solution of 0.3% NaCl was used to estimate motility. For the evaluation of motility, about 10 ml of semen were placed on a glass microscope slide and 100 ml of activation solution were added. Motility was evaluated visually using a light microscope at ×100 magnification and was expressed as percentage of motile spermatozoa. Each motility determination was performed in triplicate for each semen sample. The duration of sperm motil-

ity was estimated using a sensitive chronometer (1/100 s) until almost 90% of the sperm stopped their progressive movement. As preliminary experiments revealed the influence of pollutants on sperm motility, it was most clearly detected in the initial phase that 10±2 s (mean±S.D.) following activation.

Determination of spermatozoa density and pH. The spermatozoa density was estimated using the haemocytometric method. For this aim, a droplet of the diluted sperm was placed on a Thoma 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 ×100 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.

Effect of ions on sperm motility. Chemicals used in the study were obtained from Sigma Chem. Co. (St. Louis, MO, U.S.A.). The primary buffer used was the Hank sperm incubation solution (SIS: 120 mM of NaCl, 0.25 mM of Na_2HPO_4, 0.84 mM of KCl, 10 mM of $CaSO_4$ and 10 mM of $MgSO_4$ titrated to pH 8.5 with NaOH; osmolality adjusted to 260 mOsm kg^{-1} with NaCl using a Wescor 5520 vapour pressure osmometer; Logan, UT, U.S.A.). The SIS was modified to determine the effect of different solutions (NaCl, KCl, $CaSO_4$ and $MgSO_4$) at different concentrations (NaCl: 15, 25, 35, 45 mM; KCl: 0.5, 1, 1.5, 2 mM; $CaSO_4$: 2.5, 5, 7.5, 10; $MgSO_4$: 5, 10, 15, 20 mM) on motility. In addition, to test the effect of different ions, the SIS was modified to contain various concentrations of Na^+ (25, 50, 100 and 125 mM), K^+ (0.2, 0.5, 2 and 5 mM), Ca^{2+} (1, 5, 10 and 15 mM) and Mg^{2+} (3, 5, 10 and 15 mM). Sperm motility was assessed on contact with SIS (dilution 1:1000) and following 1-hour incubation on dilution (1:10) with distilled water.

Effect of pH on sperm motility. The influence of pH on sperm motility was determined in doubly distilled water which was buffered to pH 5.0, 6.0, 7.0, 8.0 and 9.0 with 0.1 mmol l^{-1} Hepes (pH range 5–6) or 0.1 mmol l^{-1} Tris (pH range 7–9). Each pH determination was repeated three times.

Statistical analysis. Sperm quality parameters from individual fish were analysed using one-way analysis of variance (ANOVA), followed by the Duncan new multiple range test (DNMR) with a minimum significance of $p<0.05$. Motility data were normalised through arcsine transformation. Results are presented as means±SEM. Statistical analyses were performed with the SPSS 10 for Windows statistical software package.

RESULTS AND DISCUSSION

Spermatological parameters of the collected fresh sperm were found to be rather variable and are presented in Table 1. The sperm volume collected for each male

ranged between 7.2 and 20.6 ml and the mean was found as 12.37±2.25 ml. Fresh sperm motility showed high motility and longer motility durations when diluted with distilled water. Sperm was found to be viscous in consistency and creamy white in colour in all samples.

Table 1. Spermatological parameters of scaly carp (*Cyprinus carpio*) (*n*=15).

	Minimum	Maximum	Mean	SEM
Volume (ml)	7.2	20.6	12.37	2.25
Motility (%)	70	95	85	0.50
Movement duration (s)	30	168	72	1.25
Density ($\times 10^9$/ml)	16.250	43.600	32.200	540.25
Total density ($\times 10^9$)	117.000	898.160	498.750	1246.50
pH	6.2	8.9	7.27	0.25

Maximum spermatozoa motility values were observed in solutions containing 25 mM of NaCl (95.0±16.0%), 0.5 mM of KCl (85.0±2.45%), 5 mM of $CaSO_4$ (90.0±2.27%) and 10 mM of $MgSO_4$ (80.16±2.45%) (Fig. 1 *a–d*). To assess the influence of Na^+ on sperm motility sperm samples were incubated for 1 h in SIS containing various concentrations of Na^+ (25, 50, 100 and 125 mM). Sperm incubated in SIS containing 50 mM and higher concentrations of Na^+ exhibited low motility (<40) when diluted in distilled water. Sperm diluted in modified SIS containing 2 mM and higher concentrations of K^+ exhibited <30% motility on initial contact with distilled water. Motility of sperm, incubated in SIS containing Ca^{2+} decreased significantly with increasing concentrations of Ca^{2+} (>10 mM) when sperm were diluted in distilled water ($p<0.05$). Sperm samples incubated in SIS containing 3 and 5 mM of Mg^{2+} displayed high motility following dilution in distiled water ($p<0.05$) (Fig. 2 *a–d*). The highest total duration of sperm motility was observed in solutions containing 25 mM of NaCl (147.0±5.27 s), 0.5 mM of KCl (174±2.47 s), 5 mM of $CaSO_4$ (95.2±3.46 s) and 10 mM of $MgSO_4$ (124.56± 26.42 s) (Fig. 3 *a–d*). The sperm motility rate decreased at >pH 8.0 and <pH 6.0 (Fig. 4).

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. In general, sperm must be motile to achieve fertilisation, and low fertility rates are correlated with sperm samples that contain low percentages of motile sperm[8]. In the present study, mean sperm motility was 75±2.35%. Motility varies in vigour and duration not only among males but also within an individual male depending on ripeness[9]. Most studies on fish species have shown that the duration and motility of semen can vary seasonally[10]. The differences may be due to feeding conditions, age, environmental factors, time of spawning, dilution ratio, and ionic composition of the seminal plasma.

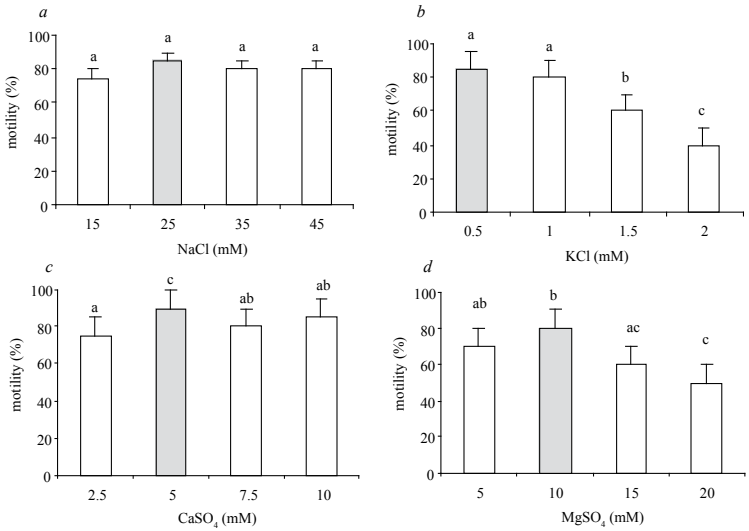

Fig. 1. Sperm motility of scaly carp following dilution with sperm incubation solution (SIS) containing *a* – 15, 25, 35 and 45 mM of NaCl; *b* – 0.5, 1, 1.5 and 2 mM of KCl; *c* – 2.5, 5, 7.5 and 10 mM of $CaSO_4$; *d* – 5, 10, 15, 20 mM of $MgSO_4$. Treatments with same lowercase letter are not significantly different ($p< 0.05$, $n=3$)

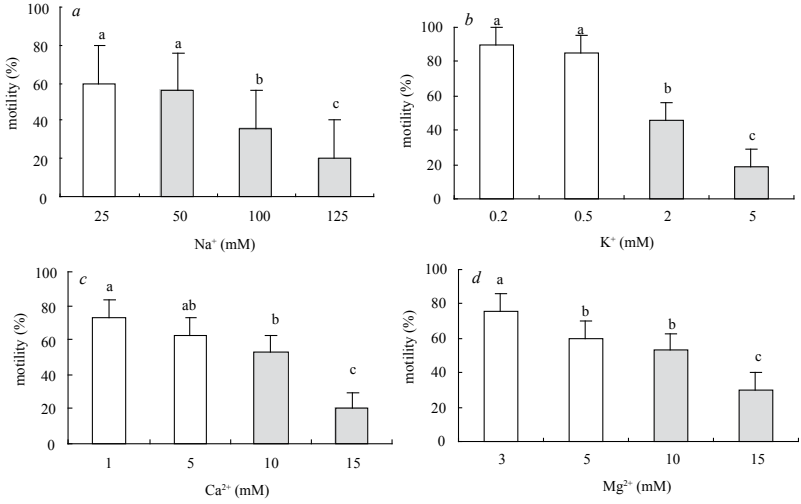

Fig. 2. Sperm motility of scaly carp following dilution with sperm incubation solution (SIS) containing *a* – 25, 50, 100 and 125 mM of Na^+; *b* – 0.2, 0.5, 2 and 5 mM of K^+; *c* – 1, 5, 10 and 15 mM of Ca^{2+}; *d* – 3, 5, 10 and 15 mM of Mg^{2+}. Treatments with same lowercase letter are not significantly different ($p< 0.05$, $n=3$)

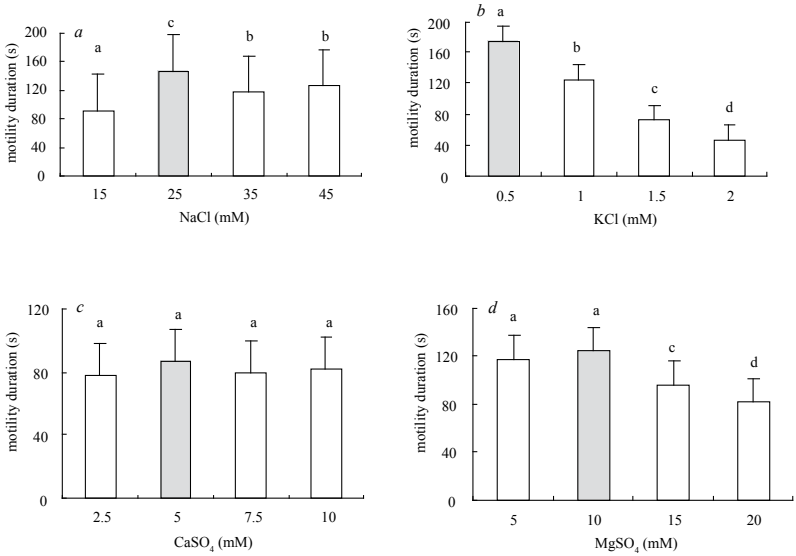

Fig. 3. Sperm motility durations of scaly carp following dilution with sperm incubation solution (SIS) containing *a* – 15, 25, 35 and 45 mM of NaCl; *b* – 0.5, 1, 1.5 and 2 mM of KCl; *c* – 2.5, 5, 7.5 and 10 mM of $CaSO_4$; *d* – 5, 10, 15, 20 mM of $MgSO_4$. Treatments with same lowercase letter are not significantly different ($p<0.05$, $n=3$)

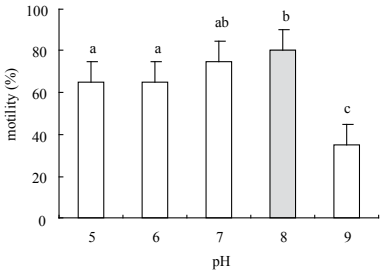

Fig. 4. Sperm motility sensitivity of scaly carp to pH. Sperm motility following dilution with sperm incubation solution (SIS) containing 12.5 mM of KCl, adjusted to pH 5.0, 6.0, 7.0, 8.0 and 9.0. Treatments with same lowercase letter are not significantly different ($p<0.05$, $n=3$)

In the present study spermatozoa were not separated from the seminal plasma but the whole semen was prediluted in sperm motility inhibiting solution (SIS). This is similar to the natural spawning conditions where spermatozoa are also released together with seminal fluid into the aquatic environment. Inorganic constituents such as Na^+, K^+, Ca^{2+} and Mg^{2+} are involved in the process of inhibition or activation of sperm motility[11]. Different actions of environmental pollutants on spermatozoa are possible as the processes of motility activation and regulation are complex[12]. The chemicals might act on proteins of the plasma membrane or on ion channels.

As the spermatozoal membrane of teleosts is highly permeable to low molecular substances, the chemicals may also enter into the sperm cell[13].

Sperm diluted in SIS containing >50 mmol l^{-1} of Na^+ expressed and decrease motility compared to the lower concentrations of Na^+. It suggests that high Na^+ concentration has a slight inhibitory influence on sperm motility. Such a finding is consistent with inhibitory effect of Na^+ in sperm of other species[14]. Therefore, sperm exposed to Na^+ were activated either on contact or during incubation despite the high osmolality SIS (260 mOsm kg^{-1}). Similarly, when sperm were incubated with a relatively high concentration of exogenous K^+ (>2 mmol l^{-1}), sperm did not become motile. Also, sperm diluted in SIS containing >10 mmol l^{-1} of Ca^{2+} and 10 mmol l^{-1} of Mg^{2+} showed a decrease in spermatozoa motility. The findings of this research showed that high cooncentrations of inorganic ion components have important role to onset of sperm motility.

The sperm motility of *Cyprinus carpio* was affected by pH. The sperm motility rate decreased at pH > 8.0 and pH < 6.0. Therefore, the spermatozoa were less tolerant to acidic and alkaline pH than spermatozoa of *Salmo trutta fario*, *Leuciscus cephalus* and *Lota lota* where sperm motility was affected by pH < 6.0 and than spermatozoa of *Clarias gariepinus* where sperm motility was affected by pH > 9.0 (Ref. 13). A decrease in sperm motility at acid or alkaline pH is related to changes in intraspermatozoa ion concentrations which alter the membrane potential that is necessary for motility activation. In unbuffered motility activating solutions the inorganic pollutants may change the pH depending on their pH values[15]. Therefore, changes in sperm motility behaviour might be an effect of pH but not of the test substances. This was prevented by the experimental design. Although distilled water was used for motility activation, the whole sperm suspension had a buffering capacity derived from the sperm motility inhibiting saline solution which kept the pH at a relative constant range.

Medium pH has little impact on carp spermatozoa motility except at extreme pH, via an Na+/H+ exchanger[16]. Findings of Redondo-Müller et al. [17] have been supporting the present results that carp spermatozoa motility can be initiated in medium with pH between 6.0 and 9.0. Activation of carp spermatozoa appears independent of the pH since the addition of NH_4Cl does not trigger sperm motility, in contrast to sea urchin spermatozoa[18]. Alteration of the internal pH (pHi) as possible mechanism interfering with motility was investigated on spermatozoa from different species. As examples, it has been shown on trout spermatozoa that change in the pH value induces a change in the pH (Ref. 19). In this regard, it was observed that the intracellular alkalinisation (0.15 pH units) which accompanied the motility initiation of carp sperm does not play a key role to trigger axonemal movement[20]. Similar results were also determined for trout (*Salmo* sp.) [19]. Obviously, intracellular pH is one important factor to regulate fish sperm motility as reported in many other organisms. Recently, motility experiments in the presence

of nigericin have shown that intracellular pH at values higher than 7.0 are necessary to induce the sperm motility of flat fish species[21].

In addition, the recent studies showed that the accumulation of pollutants in the fish is a very critical factor as it can negatively affect the process of spermiogenesis and oogenesis and disrupt of reproduction[22]. Current data suggest that the ionic composition of the dilution medium is an important factor to initiate sperm motility. Also, this results suggest that the regulation of sperm motility by the dilution medium is not simply an osmotic phenomenon, although the osmotic strength of the solution has a role in the initiation of motility[1].

CONCLUSIONS

The results of the present study demonstrated that the nature of the pre-activation environment as well as the composition of the activation medium can have significant effects on sperm motility. Thus, during natural spawning, the physiological state of the male before sperm release as well as the chemical composition of the microenvironment near the egg micropyle are likely to influence sperm motility and may infuence fertility.

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