Relationships between seminal plasma composition and sperm quality parameters of the *Salmo trutta macrostigma* (Dumeril, 1858) semen: with emphasis on sperm motility

Y. Bozkurt[1], F. Öğretmen[2], Ö. Kökçü[3] U. Erçin[4]

[1]Department of Aquaculture, Faculty of Fisheries, Mustafa Kemal University, Hatay, Turkey
[2]State Hydraulic Works, Fish Production Station, Adana, Turkey
[3]Environment Conservation and National Parks Fish Production Station, Mersin, Turkey
[4]Department of Biochemistry, Faculty of Medicine, Gazi University, Ankara, Turkey

ABSTRACT: The mineral and organic composition of seminal plasma, physical spermatological parameters and their physiological relationships were investigated in *Salmo trutta macrostigma*. The seminal plasma contained 121.0 ± 0.37mM/l (Na^+), 8.18 ± 0.03mM/l (K^+), 7.23 ± 0.03 mg/dl (Ca^{2+}), 3.19 ± 0.02 mEq/l (Mg^{++}), 0.48 ± 0.02 g/dl total protein, 6.07 ± 0.06 mg/dl cholesterol, 6.24 ± 0.08 mg/dl triglyceride and 9.97 ± 0.39 mg/dl urea. The following physical spermatological parameters were found out: sperm volume 13.93 ± 0.84 ml, sperm motility 80.37 ± 2.36%, movement duration 81.47 ± 4.21 s, density 6.02 ± 0.46 × 10^9/ml, total density 8.85 ± 6.12 × 10^9 and pH 7.53 ± 0.20. Significant positive relationships were determined between motility duration and motility (r = 0.83, P < 0.01) and also between spermatocrit and motility (r = 0.536, P < 0.05). Sperm volume and total density negatively correlated with motility (r = –0.191, P > 0.05 and r = –0.087, P > 0.05, respectively). The Na^+, K^+ and Cl^- ions correlated negatively with motility (r = –0.267, P > 0.05, r = –0.152, P > 0.05 and r = –0.461, P > 0.05, respectively). On the other hand, the Ca^{2+} and Mg^{2+} ions correlated positively with motility (r = 0.114, P > 0.05 and r = 0.040, P > 0.05, respectively). A significant negative relationship was found between motility and urea (r = –0.515, P < 0.05). These parameters should be considered when developing procedures for short-term storage or cryopreservation of the *Salmo trutta macrostigma* sperm.

Keywords: spermatological parameters; spermatozoa; seminal fluid; *Salmo trutta macrostigma*

Salmo trutta macrostigma (Dumeril, 1858) is a salmonid species occurring in inland water habitats of Southern Europe, Western Asia, Northern Africa, and Anatolia (Geldiay and Balik, 1988). It is also a critically endangered fish species in Turkish inland waters because of illegal fishing, overfishing, and other environmental changes, including hydroelectric power plants and pollution. For this reason, *Salmo trutta macrostigma* has been considered for a biological conservation program in Turkey.

Evaluation of sperm quality provides necessary information for aquaculturists to devise optimal handling and storage protocols for sperm used in artificial fertilization (Billard et al., 1995; Linhart et al., 2004). The use of high-quality gametes from captive fish brood stock is of great importance to ensure the valuable offspring production in aquaculture (Bromage, 1995; Hajirezaee et al., 2010). In order to provide controlled and successful production in aquaculture systems, the knowledge of

physical and chemical characteristics of fish semen is very important.

Motility is the most commonly used parameter to evaluate sperm quality in fish (Billard et al., 1995; Lahnsteiner and Patzner, 1998). In general, spermatozoa must be motile to achieve fertilization since low fertility rates are correlated with sperm samples that show a low percentage of motility. The sperm density has traditionally been used for the assessment of semen quality. It is an important parameter which has an impact on fertilization success and is a characteristic feature of fish species (Agarwal, 2005). The standard method of sperm cell counting using a haemocytometer opted by most of the earlier workers is time-consuming. It loses its significance when the quick estimation of sperm count in unit volume of semen samples is required at the time of artificial fertilization or semen cryopreservation. Therefore, optional methods like centrifugation of semen to determine spermatocrit have been employed to rapidly determine sperm density (Glogowski et al., 1999; Rakitin et al., 1999).

The composition of seminal plasma has a great influence on the biological quality of the semen. Determination of seminal plasma composition can help to understand the design requirements to prepare the appropriate artificial seminal plasma solutions. Such solutions can be used for the dilution of semen for short-term storage or cryopreservation (Billard and Cosson, 1992; Dreanno et al., 1998). In addition, seminal plasma is an important constituent of semen that has a vital role in sperm metabolism, function, survival and sperm motility. The ions such as Na^+, K^+ and Cl^- in the seminal plasma establish osmotic balance.

Seminal plasma composition in salmonids has been carefully studied particularly in rainbow trout and salmon (Piironen and Hyvarinen, 1983; Munikittrick and Moccia, 1987; Lahnsteiner et al., 1998; Glogowski et al., 2000). The composition of seminal plasma in other salmonid species has been examined scarcely so far (Cruea, 1969; Morisawa et al., 1979; Piironen and Hyvarinen, 1983; Hatef et al., 2007). It is well known that the composition of seminal plasma can differ even among related species.

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 seminal plasma. Another objective was (c) to study the physiological relationships between the sperm motility and the mineral and organic contents of seminal plasma in order to establish species-specific characteristics and expand our knowledge of *Salmo trutta macrostigma* semen.

MATERIAL AND METHODS

Broodstock care and collection of semen

Mature males were obtained from wild-caught broodstock held at the Environment Conservation and National Parks Fish Production Station (Çamliyayla, Mersin). Water was supplied to the station at a flow rate of 1.5 l/s. The water temperature was 8.4 ± 0.2°C and broodstock were fed with a pelleted diet (45% protein). The maximal fish density was 5–6 kg/m^3. Fifteen mature *Salmo trutta macrostigma* males (total weight 2.73 ± 0.54 kg, total length 42.61 ± 1.27 cm) were randomly selected from the broodstock for use as semen donors. The fish were not fed 48 h prior to the sperm collection. Each male was stripped once only and the total amount of expressible milt was collected individually by gently pressing the abdomen. The semen was collected directly into clean and dry glass tubes. Care was taken to avoid the contamination of semen with water, urine, blood or faecal matter. The tubes were covered and immediately transported on ice (4°C) to the laboratory for analyses.

Evaluation of semen volume, pH and colour

Sperm was sampled into 20-ml calibrated glass tubes and the volume was expressed as ml. Sperm pH was measured using standard pH-electrodes within 30 min of sampling. Semen colour was evaluated visually following the semen collection.

Evaluation of sperm motility

The motility of sperm in each sample was evaluated within the first hour following the semen collection. For this aim 10 µl semen was pipetted onto a 1% (w/v) BSA-coated microscope slide. Activation was achieved by adding 25 µl of 0.3% NaCl solution. Motility observation was carried out under a prefocused inverted microscope (400x, Olympus CK2, Tokyo, Japan) at 10°C. Only forward movements of spermatozoa were assessed as motile whereas simply vibrating spermatozoa were assessed as immobile.

The percentage of motility was determined arbitrarily on a 0 to 10 point scale, 0 denoting 0% motility and 10 denoting 100% motility. Once diluted, the semen was held on ice between preparations of replicate slides. The duration of motility was determined by recording the time taken from activation to the complete cessation of activity of the last spermatozoa in that field. Sperm motility observations were done using three replicates per sample. One person conducted all the sperm motility observations in order to decrease the degree of variation.

Evaluation of sperm density and spermatocrit

Sperm density was determined according to the haemacytometric method. The sperm was diluted at a ratio of 1:1000 with Hayem's solution (5 g Na_2SO_4, 1 g NaCl, 0.5 g $HgCl_2$ and 200 ml bidistilled water). A droplet of the diluted milt was placed on a haemocytometer slide (depth 0.1 mm) with a coverslip and counted using light microscopy. Counting chambers were kept in moist atmosphere (to allow sperm sedimentation) for 10 min at least before cell counting. Then, the number of spermatozoa was counted in 16 cells and calculated according to Caille et al. (2006). Spermatozoa density was expressed as $\times 10^9$/ml.

For spermatocrit measurement, micro-haematocrit capillary tubes (75 mm length, 1 mm inner diameter and 0.1 ml capacity) were filled with semen and their both ends were sealed with haemoseal wax. The volume (length) of semen in capillaries was measured by meter scale in mm and centrifuged for 10 min at 4000 rpm (3370× g). Spermatocrit is defined as the percentage volume of white packed cells to the total volume of semen. Measurements were done in triplicate for each sample and the average of three measurements was used in subsequent statistical analyses. Spermatocrit was measured within 1 h of the semen collection.

Evaluation of seminal plasma composition

Seminal plasma was collected after centrifugation (Heraeus, Labofuge 200, Germany) of the semen at 3370× g for 10 min. Seminal plasma was centrifuged twice to avoid possible contamination with spermatozoa and stored in Eppendorf (Wiesbaden, Germany) vials at –20°C until the beginning of analysis. 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

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

Spermatological parameters

Spermatological parameters of the collected sperm were found rather variable and they are shown in Table 1. The sperm motility ranged be-

Table 1. Spermatological parameters of *Salmo trutta macrostigma* (n = 15)

	Minimum	Maximum	Mean	SEM
Volume (ml)	8	19	13.93	0.84
Motility (%)	60	95	80.37	2.36
Movement duration (s)	47	105	81.47	4.21
Density ($\times 10^9$/ml)	3.24	8.60	6.02	0.46
Total density ($\times 10^9$)	45.30	137.60	80.85	6.12
Spermatocrit (%)	24	72	55.6	3.80
pH	6	9	7.53	0.20

tween 60% and 95% and the mean was 80.37 ± 2.36%. Sperm motility durations were determined ranging from 47 s to 105 s with an average of 81.47 ± 4.21 s. Motility duration and motility significantly correlated with each other (r = 0.830, P < 0.01). Sperm motility and urea were negatively correlated (r = –0.515, P < 0.05). The sperm volume collected for each male ranged between 8.0 and 19.0 ml and the mean was 13.93 ± 0.84 ml. Sperm volume showed a negative allometry with sperm density (r = –0.555, P < 0.05).

On the other hand, sperm density correlated positively with total sperm density (r = 0.601, P < 0.05). Spermatocrit value was determined to range from 24 to 72% with an average of 55.6 ± 3.80%. Spermatocrit value positively correlated with density (r = 0.661, P < 0.01), motility (r = 0.536, P < 0.05) and cholesterol (r = 0.545). On the other hand, spermatocrit value negatively correlated with volume (r = –0.584, P < 0.05) and chlorine (r = –0.623, P < 0.05). It was observed that the spermatocrit value decreased as the viscosity (thickness) of the semen was reduced. The sperm was found to be viscous in consistency and creamy white in colour in all samples.

Seminal plasma composition and spermatocrit

The composition of seminal plasma ions and metabolites is shown in Table 2.

The ionic content of seminal plasma was found rather variable. The Na^+ ion correlated positively (r = 0.705, P < 0.01) with K^+ ion. The Ca^{2+} ion correlated negatively with total sperm density and urea (r = –0.615, P < 0.05 and r = –0.633, P < 0.05, respectively). Similarly, K^+ and Cl^- ions were negatively correlated with cholesterol (r = –0.590, P < 0.05 and r = –0.600, P < 0.05, respectively). On the other hand, Cl^- ion was positively correlated with urea (r = 0.524, P < 0.05). Also, pH was significantly positively correlated with total protein (r = 0.587, P < 0.05). The relationships between biochemical and physical spermatological parameters are shown in Table 3.

The Na^+ and K^+ ions correlated negatively with sperm motility (r = –0.267, P < 0.05 and r = –0.152, P < 0.05, respectively) (Figure 1a and b). Also, a negative allometry was found between sperm motility and Cl^- (r = –0.461, P < 0.05) (Figure 1e). On the other hand, Ca^{2+} and Mg^{2+} ions correlated positively with sperm motility (r = 0.114, P > 0.05 and r = 0.040, P > 0.05) (Figure 1c and d). Sperm motility positively correlated with protein, triglyceride and cholesterol (r = 0.053, P > 0.05, r = 0.040, P > 0.05 and r = 0.318, P > 0.05) (Figure 1f–h). But, motility negatively correlated with urea (r = –0.515, P < 0.05) (Figure 1i).

DISCUSSION

The composition of fish semen and its physical characteristics have been found to vary with the species and are important from the aspect of semen quality (Kruger et al., 2006). The semen quality also varies considerably among the individuals of the same species (Piironen, 1985). The semen quality, particularly in aquaculture species,

Table 2. Seminal plasma ion and metabolite composition of *Salmo trutta macrostigma* sperm (n = 15)

	Minimum	Maximum	Mean	SEM
Na^+ (mM/l)	119	123	121	0.37
K^+ (mM/l)	8	8.4	8.18	0.03
Ca^{2+} (mg/dl)	7	7.4	7.23	0.03
Mg^{2+} (mEq/l)	3.1	3.3	3.19	0.02
Cl^- (mM/l)	124	128	125.60	0.36
Total Protein (g/dl)	0.4	0.6	0.48	0.02
Triglyceride (mg/dl)	6.2	6.3	6.24	0.08
Cholesterol (mg/dl)	5.7	6.4	6.07	0.06
Urea (mg/dl)	8.56	12.84	9.97	0.39

Table 3. Correlations between spermatological parameters and seminal plasma composition of *Salmo trutta macrostigma* sperm

	Volume	Motility	Movement duration	Density	Total density	Spermatocrit	pH	Ca^{2+}	Na^{+}	K^{+}	Mg^{2+}	Cl^{-}	Protein	Triglyceride	Cholesterol
Motility	–0.191														
Movement duration	–0.182	0.830**													
Density	–0.555*	0.211	0.191												
Total density	0.309	–0.087	–0.042	0.601*											
Spermatocrit	–0.584*	0.536*	0.500	0.661**	0.191										
pH	–0.013	0.311	0.167	–0.171	–0.231	0.085									
Ca^{2+}	–0.286	0.114	–0.023	–0.270	–0.615	–0.036	0.506								
Na^{+}	0.329	–0.267	–0.472	–0.248	0.004	–0.365	0.330	0.038							
K^{+}	0.226	–0.152	–0.211	–0.213	–0.023	–0.311	0.287	0.069	0.705**						
Mg^{2+}	–0.150	0.040	–0.028	–0.003	–0.167	0.083	0.386	0.447	0.094	–0.229					
Cl^{-}	0.430	–0.461	–0.399	–0.422	–0.050	–0.623*	–0.332	–0.254	0.348	0.416	–0.118				
Protein	–0.397	0.053	–0.048	0.181	–0.206	0.019	0.587*	0.504	0.117	–0.156	0.487	–0.476			
Triglyceride	–0.008	0.040	0.153	0.367	0.415	0.333	–0.380	–0.094	–0.409	–0.359	0.018	–0.102	–0.288		
Cholesterol	–0.510	0.318	0.418	0.430	0.022	0.545*	0.182	–0.085	–0.576*	–0.590*	0.174	–0.600*	0.469	0.044	
Urea	0.064	–0.515*	–0.277	0.034	0.220	–0.074	–0.240	–0.633*	0.279	0.299	–0.195	0.524*	–0.356	–0.074	–0.052

*Significant at $P < 0.05$

**Significant at $P < 0.01$

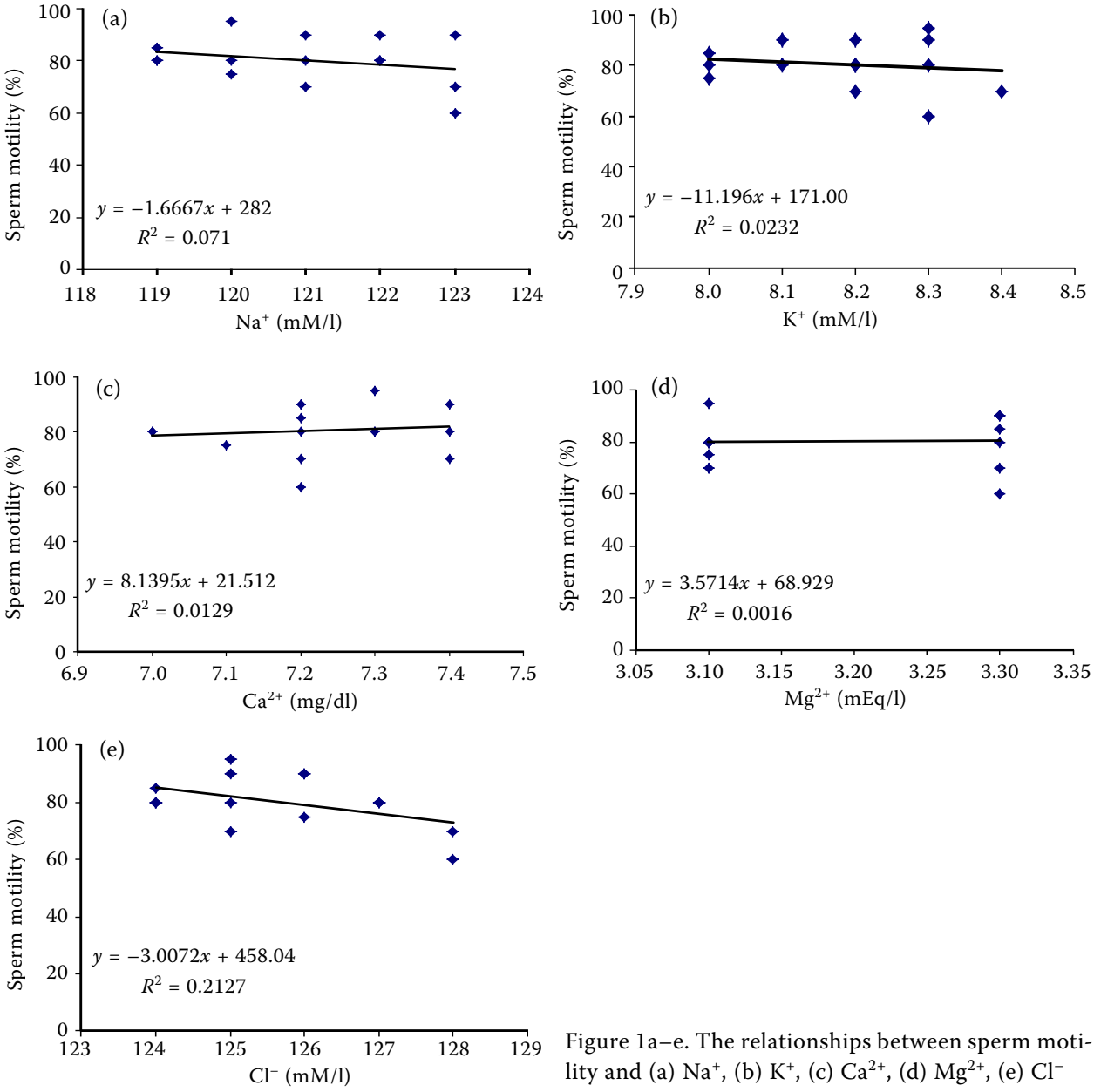

Figure 1a–e. The relationships between sperm motility and (a) Na^+, (b) K^+, (c) Ca^{2+}, (d) Mg^{2+}, (e) Cl^-

depends on various external factors such as feeding regime, feed quality, rearing temperature and spawning season of males (Bromage and Roberts, 1995; Rurangwa et al., 2004). In the present study, the mean sperm motility was determined to be 80.37 ± 2.36%. Motility varies in vigour and duration depending on the ripeness of male fish (Tekin et al., 2003). Most studies on fish species have shown that the duration and motility of spermatozoa can vary seasonally (Benau and Terner, 1980; Akcay et al., 2004). The differences may be due to differences in feeding conditions, age, environmental factors, spawning time, dilution ratio, and ionic composition of the seminal plasma (Bozkurt et al., 2009a).

The ionic composition of seminal plasma has a significant influence on sperm motility in fish. In salmonids, the motility of spermatozoa is mainly controlled by the K^+ concentration. It is generally known that a higher K^+ concentration inhibits sperm motility in salmonids (Morisawa and Suzuki, 1980) but it increases sperm motility in carp (Billard and Cosson, 1992, Bozkurt et al., 2009b). In our study, the K^+ concentration (8.18 ± 0.03mM/l) was lower than in Atlantic salmon (28mM/l; Aas et al., 1991), *Salmo trutta abanticus* (38mM/l; Bozkurt, 2008a),

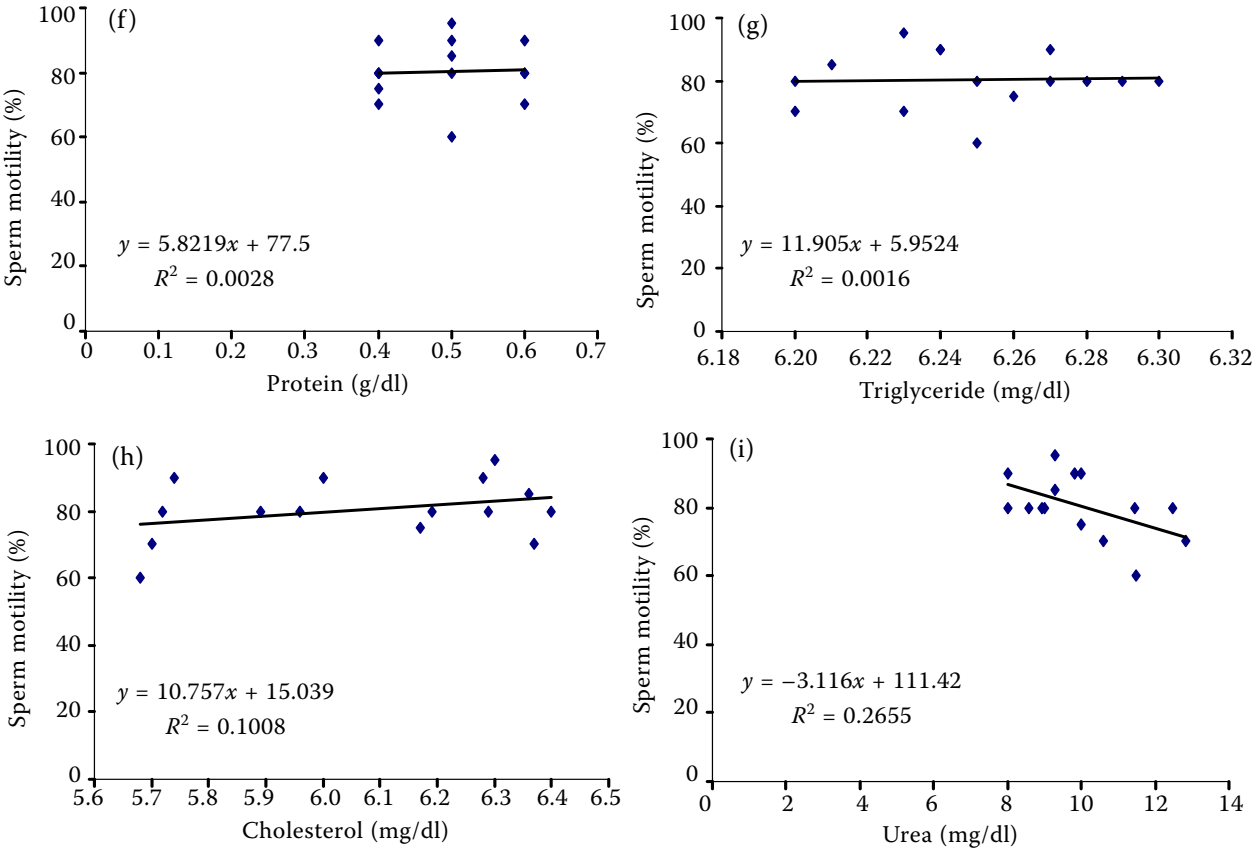

Figure 1f–i. The relationships between sperm motility and (f) protein, (g) triglyceride, (h) cholesterol, (i) urea

rainbow trout (46mM/l; Secer et al., 2004), perch (10 mM/l; Lahnsteiner et al., 1995), and Asian catfish (18 mmol/l; Tan-Fermin et al., 1999). It can be concluded that the K^+ ion at certain concentrations also tends to inhibit the *Salmo trutta macrostigma* spermatozoa since a negative allometry was determined between K^+ and motility.

The seminal plasma of *Salmo trutta macrostigma* has a higher Na^+ content (121.0 ± 0.37mM/l) than rainbow trout (80 mmol/l; Secer et al., 2004) and grass carp (98 mmol/l; Bozkurt et al., 2008b) but lower than other freshwater species such as 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). It is interesting to note that there is also a negative interaction between Na^+ and motility ($P > 0.05$) in spite of the higher Na^+ level than that reported for the rainbow trout (Secer et al., 2004). It seems that this situation can be a species-specific characteristic and the negative relationships between K^+, Na^+ and motility show that the sperm motility in *Salmo trutta macrostigma* sperm depends on other ions such as Ca^{2+} and Mg^{2+} concentration. Ca^{2+} and Mg^{2+} also contribute significantly to the ionic composition of seminal plasma in fish semen. 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 inhibition of sperm motility by K^+ can be overcome by an increased external Ca^{2+} concentration. Preliminary results indicate that the intracellular Ca^{2+} concentration increases when motility is initiated (Billard et al., 1989; Cosson et al., 1989; Boitano and Omoto, 1991). Similarly, the motility of *Salmo trutta macrostigma* spermatozoa was increasing when the Ca^{2+} and Mg^{2+} ion levels in seminal plasma were high. In addition, Billard et al. (1995) reported that external Ca^{2+} ions were necessary for motility initiation.

Compared to the other vertebrates, the quantity of proteins in seminal plasma of fish is rather low (Krol et al., 2006). In this study, the seminal plasma protein content was determined to amount to 0.48 ± 0.02 g/l, which was a similar value to *Salmo trutta*

caspius (Hatef et al., 2007). The total content of proteins in seminal plasma decreases in the second phase of spermiation (Sanchez-Rodriguez et al., 1978). It is supposed that this component may play an important protective role for spermatozoa and it contains a number of key enzymes of the metabolic process (Lahnsteiner et al., 1995; Kowalski et al., 2003). On the other hand, notable concentrations of urea (9.97 ± 0.39 mg/dl) were also determined in seminal plasma. Urea contamination of semen may cause reduced sperm motility and fertilizing ability (Dreanno et al., 1998) influencing the variability of other semen parameters (Glogowski et al., 2000).

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 (6.24 ± 0.08 mg/dl) was positively correlated with sperm motility. According to Lahnsteiner et al. (1993) triglycerides serve as an energy source for spermatozoa during immotile storage and during the regeneration phase after motility. In this study the cholesterol level was determined to be 6.07 ± 0.06 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 may have a protective effect against environmental changes (especially in temperature) that occurs when the fish semen is released (Bozkurt et al., 2008b). Another interesting point is the lack of glucose in seminal plasma. Piironen and Hyvarinen (1983) also noted a zero glucose concentration and low motility in *Salmo trutta m. lacustris*. The lack of glucose in seminal plasma can be explained by the spawning stage or contamination with bacteria that quickly decompose glucose in sperm.

The present study showed that the *Salmo trutta macrostigma* produced sperm with a very low density (6.02 ± 0.46 × 10^9/ml) compared to the other salmonid fish. For instance, sperm densities were reported in the range of 8.9–11.8 × 10^9/ml for rainbow trout (*Oncorhynchus mykiss*) (Ciereszko and Dabrowski, 1993; Glogowski et al., 2000) and 14.1 x 10^9/ml for brown trout (*Salmo trutta*) (Piironen and Hyvarinen, 1983). These differences can be related to many factors including age and weight of the male (Suquet et al., 1998), ecology and spawning behaviour of broodstock (Piironen and Hyvarinen, 1983), sampling period and spawning method (Suquet et al., 1994) and also spawning stage (Tekin et al., 2003). In the present investigation, spermatocrit value was also calculated for quick estimation of sperm density. Like in most fish species, after semen centrifugation, there exists a clearly defined interface between the packed sperm cells and the clear seminal fluid which gives true estimates of spermatocrit. A significant highly positive relationship ($r = 0.661$, $P < 0.05$) between spermatocrit and sperm density was determined in this study. A similar significant correlation between sperm density and spermatocrit was reported in rainbow trout (Baynes et al., 1981) and for several other teleost species such as Atlantic salmon (*Salmo salar*) (Piironen, 1985), rainbow trout (Ciereszko and Dabrowski, 1993) and carp (Takashima et al., 1984).

CONCLUSION

The knowledge of physical and chemical constituents of spermatozoa and seminal plasma is a prerequisite for the successful evaluation of the reproductive ability of different fish species. This may also lead to the better understanding of fertilization mechanisms. On the other hand, there are some species-specific characteristics in terms of the mineral and organic composition of seminal plasma that should be considered for artificial insemination or sperm storage. It can be concluded that the relationship determined between spermatocrit and sperm density in the *Salmo trutta macrostigma* also recommends the use of spermatocrit as a simple, fast and cheap method of determining the sperm density in this salmonid species.

In addition, the findings of this research can be used to select high-quality mature males for egg fertilization in a commercial aquaculture operation and, as a result of reducing the number of male broodstock, the economic efficiency of the farm can be increased. The information on sperm physiology obtained in the present study could lead to more efficient gamete management and increase yields, and enhance the suitability of semen for cryopreservation.

Acknowledgements

The authors would like to thank the staff of the Environment Conservation and National Parks Fish Production Station, Mersin, Turkey.

REFERENCES

Aas G.H., Refstie T., Gjerde B. (1991): Evaluation of milt quality of Atlantic salmon. Aquaculture, 95, 125–132.

Agarwal N.K. (2005): Developing cryopreservation protocols for the spermatozoa of snowtrout and other endangered coldwater species. [Final report.] ICARScheme, 105.

Akcay S.M., Scott A.P., Dawson A.P. (1981): Rainbow trout, *Salmo gairdneri* Richardson, spermatozoa: Effects of cations and pH on motility. Journal of Fish Biology, 19, 259–267.

Akcay E., Bozkurt Y., Secer S., Tekin N. (2004): Cryopreservation of mirror carp semen. Turkish Journal of Veterinary and Animal Sciences, 28, 837–843.

Baynes S.M., Scott A.P., Dawson A.P. (1981): Seasonal variation in parameters of milt production and plasma concentration of sex steroid in male rainbow trout (*Salmo gairdneri*). General and Comparative Endocrinology, 57, 150–160.

Benau D., Terner C. (1980): Initiation, prolongation and reactivation of the motility of salmonid spermatozoa. Gamete Research, 3, 247–257.

Billard R., Cosson M.P. (1992): Some problems related to the assessment of sperm motility in freshwater fish. Journal of Experimental Zoology, 261, 122–131.

Billard R., Bieniarz K., Popek W., Epler P., Saad A. (1989): Observation on a possible pheromonal stimulation of milt production in carp, *Cyprinus carpio*. Aquaculture, 77, 387–392.

Billard R., Cosson J., Crim L.W., Suquet M. (1995): Sperm physiology and quality. In: Bromage N.R., Roberts R.J. (eds): Brood Stock Management and Egg and Larval Quality. Blackwell Science, Oxford, 25–52

Boitano S., Omoto C.K. (1991): Membrane hyperpolarization activates trout sperm without an increase in intracellular pH. Journal of Cell Science, 98, 343–349.

Bozkurt Y. (2008a): Physical and biochemical properties of *Salmo trutta abanticus* semen. Indian Veterinary Journal, 85, 282–284.

Bozkurt Y., Öğretmen F., Erçin U., Yıldız U. (2008b): Seminal plasma composition and its relationship with physical spermatological parameters of grass carp (*Ctenopharyngodon idella*) semen: with emphasis on sperm motility. Aquaculture Research, 39, 1666–1672.

Bozkurt Y., Öğretmen F., Secer F.S., Erçin U. (2009a): Effects of seminal plasma composition on sperm motility in mirror carp (*Cyprinus carpio*). Israeli Journal of Aquaculture-Bamidgeh, 61, 307–314.

Bozkurt Y., Öğretmen F., Seçer F.S., Erçin U. (2009b): Relationship between seminal plasma composition and spermatological parameters in scaly carp (*Cyprinus carpio*). Journal of Animal and Veterinary Advances, 8, 2745–2749.

Bromage N.R. (1995): Broodstock management and seed quality-general considerations. In: Bromage N.R., Roberts R.J. (eds): Brood Stock Management and Egg and Larval Quality. University Press, Cambridge, 1–24.

Bromage N.R., Roberts R.J. (1995): Preservation of gametes. In: Bromage N.R., Roberts R.J. (eds): Brood Stock Management and Egg and Larval Quality. University Press, Cambridge, 53–75.

Ciereszko A., Dabrowski K. (1993): Estimation of sperm concentration of rainbow trout, white fish and yellow perch using a spectrophotometric technique. Aquaculture 109, 367–373.

Cosson M.P., Billard R., Letellier L. (1989): Rise of internal Ca^{2+} accompanies the initiation of trout sperm motility. Cell Motil Cytoskeleton, 14, 424–434.

Cruea D.D. (1969): Some chemical and physical characteristics of fish sperm. Transactions of the American Fisheries Society, 98, 785–788.

Dreanno C., Suquet M., Desbruyeres E., Cosson J., Le Delliou H., Billard R. (1998): Effect of urine on sperm quality in turbot (*Psetta maxima*). Aquaculture, 169, 247–262.

Geldiay R., Balik S. (1988): Freshwater fish in Turkey. Ege Univiversity. Fen Faculty Kitaplar Serisi, 97, 519. (in Turkish)

Glogowski J., Babiak I., Kucharczyk D., Luczynski M., Piros B. (1999): Some properties of bream *Abramis brama* L. sperm and its cryopreservation. Aquaculture Research, 30, 765–772.

Glogowski J., Kwasnik M., Piros B., Dabrowski K., Goryczko K., Dobosz S., Kuzminski H., Ciereszko A. (2000): Characterization of rainbow trout milt collected with a catheter: semen parameters and cryopreservation success. Aquaculture Research, 31, 289–296.

Hajirezaee S., Mojazi Amiri B., Mirvaghefi A., Sheikh Ahmadi A. (2010): Evaluation of semen quality of endangered Caspian brown trout (*Salmo trutta caspius*) in different times of spermiation during the spawning season. Czech Journal of Animal Scinces, 55, 445–455.

Hatef A., Niksirat H., Mojazi A.B., Alavi S. M. H., Karami M. (2007): Sperm density, seminal plasma composition and their physiological relationship in the endangered Caspian brown trout (*Salmo trutta caspius*). Aquaculture Research, 38, 1175–1181.

Kowalski R., Glogowski J., Kucharczyk D., Goryczko K., Dobosz S., Ciereszko A. (2003): Proteolitic activity and electrophoretic profiles of proteases from seminal plasma of teleosts. Journal of Fish Biology, 63, 1–12.

Krol J., Glogowski J., Demska-Zakes K., Hliwa P. (2006): Quality of semen and histological analysis of testes in

Eurasian perch *Perca fluviatilis* L. during a spawning period. Czech Journal of Animal Scince, 51, 220–226.

Kruger J.C.D., Smit G.L., Van Vuren J.H.J., Ferreira J.T. (2006): Some chemical and physical characteristics of the semen of *Cyprinus carpio* L. and *Oreochromis mossambicus* (Peters). Journal of Fish Biology, 24, 263–272.

Lahnsteiner F., Patzner R.A. (1998): Sperm motility of the marine teleosts *Boops boops*, *Diplodus sargus*, *Mullus barbatus* and *Trachurus mediterraneus*. Journal of Fish Biology, 52, 726–742.

Lahnsteiner F., Patzner R.A., Weismann T. (1993): Energy resources of spermatozoa of the rainbow trout, *Oncorhynchus mykiss*. (Pisces, Teleostei). Reproduction Nutrition Development, 33, 349–360.

Lahnsteiner F., Berger B., Weismann T., Patzner R.A. (1995). Fine structure and motility of spermatozoa and composition of the seminal plasma in the perch. Journal of Fish Biology, 47, 492–508.

Lahnsteiner F., Berger B., Weismann T., Patzner R.A. (1998): Determination of sperm quality of rainbow *Oncorhynchus mykiss*, by sperm motility, seminal plasma parameters, and spermatozoal metabolism. Aquaculture, 163, 163–181.

Lin F., Liu L., Dabrowski K. (1996): Characteristics of muskellunge spermatozoa: I. Ultrastructure of spermatozoa and biochemical composition of semen. Transactions of the American Fisheries Society, 125, 187–194.

Linhart O., Rodina M., Gela D., Kocour M. (2004): Optimalization of artificial propagation in European catfish, *Silurus glanis* L. Aquaculture, 235, 619–632.

Morisawa M., Suzuki K. (1980). Osmolality and potassium ions: their roles in initiation of sperm motility in teleosts. Science, 210, 1145–1147.

Morisawa M., Hirano T., Suzuki K. (1979): Changes in blood and seminal plasma composition of the mature salmon, (*O. keta*), during adaptation to fresh water. Comparative Biochemistry and Physiolology, 64, 325–329.

Piironen J. (1985): Variation in the properties of milt from the Finnish landlocked salmon (*Salmo salarn* Sebago Girard) during the spawning season. Aquaculture, 48, 337–350.

Piironen J. (1994). Composition and cryopreservation of sperm from some Finnish freshwater teleost fish. Finnish Fisheries Research, 15, 27–48.

Piironen J., Hyvarinen H. (1983): Composition of the milt of some teleost fishes. Journal of Fish Biology, 22, 351–361.

Rakitin A., Fegruson M.M., Trippel E.A. (1999): Spermatocrit and spermatozoa density in Atlantic cod (*Codus morhua*): correlation and variation during the spawning season. Aquaculture, 170, 349–358.

Rurangwa E., Kime D.E., Ollevier F., Nash J.P. (2004): The measurement of sperm motility and factors affecting sperm quality in cultured fish. Aquaculture, 234, 1–28.

Sanchez-Rodriguez S., Escaffre A.M., Marlot S., Reinaud P. (1978): The spermiation period in the rainbow trout (*Salmo gairdneri*). Plasma gonadotropin and androgen levels, sperm production and biochemical changes in the seminal fluid. Annals of Biology, Animal Biochemistry and Biophysics, 18, 943–948.

Secer S., Tekin N., Bozkurt Y., Bukan N., Akçay E. (2004): Correlation between biochemical and spermatological parameters in rainbow trout (*Oncorhynchus mykiss*) semen. Israeli Journal of Aquaculture-Bamidgeh, 56, 274–280.

Suquet M., Billard R., Cosson J., Dorange G., Chauvaud L., Mugnier C., Fauvel C. (1994): Sperm features in turbot (*Scophthalmus maximus*): A comparison with other freshwater and marine fish species. Aquatic Living Resourses, 7, 283–294.

Suquet M., Dreanno C., Dorange G., Normant Y., Quemener L., Gaignon J.L., Billard R. (1998): The ageing phenomenon of turbot, *Scophthalmus maximus*, spermatozoa: Effects on morphology, motility and concentration, intracellular ATP content, fertilization and storage capacities. Journal of Fish Biology, 32, 31–41.

Takashima F., Weil C., Billard R., Crim L.W., Fostier A. (1984): Stimulation of spermiation by LHRH analogue in carp. Bulletin of the Japanese Society of Scientific Fisheries, 50, 1323–1329.

Tan-Fermin J.D., Miura T., Adachi S., Yamauchi K. (1999): Seminal plasma composition, sperm motility and milt dilution in the Asian catfish *Clarias macrocephalus* (Gunther). Aquaculture, 171, 323–338.

Tekin N., Secer S., Akçay E., Bozkurt Y., Kayam S. (2003): The effect of age on spermatological properties in rainbow trout (*Oncorhynchus mykiss* W. 1792). Turkish Journal of Vetetrinary and Animal Scinces, 27, 37–44.

Received: 2010–11–01
Accepted after corrections: 2011–02–08

Corresponding Author

Dr. Yusuf Bozkurt, Ph.D., Mustafa Kemal University, Faculty of Fisheries, Department of Aquaculture, 31200 Hatay, Turkey
Tel. +90 326 6141693, 206, e-mail: yfbozkurt@yahoo.com