CORRELATION BETWEEN BIOCHEMICAL AND SPERMATOLOGICAL PARAMETERS IN RAINBOW TROUT *(ONCORHYNCHUS MYKISS)* SEMEN

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Abstract

Levels of biochemical parameters in seminal plasma and physical parameters of rainbow trout (*Oncorhynchus mykiss*) semen were determined and compared. Seminal plasma contained 80.51±31.48 mmol/l Na^+, 46.21±12.58 mmol/l K^+, 4.65±1.57 mg/dl Ca^{2+}, 3.48±1.18 mEq/l Mg^{2+}, 112.5±25.93 mmol/l Cl^-, 1.33±0.76 mg/dl glucose, 0.15±0.09 g/dl protein, 2.55±2.47 mg/dl cholesterol, 8.0±2.84 mg/dl triglyceride, and 31.65±40.78 mg/dl urea. Semen volume was 19.9±21.43 ml, spermatozoa motility 60.0±30.09%, duration of spermatozoa movement 174.0±1.82 s, spermatozoa concentration 1.52±0.70 x 10^9/ml, total spermatozoa number 44.25±64.59 x 10^9, and pH 6.7±0.25.

There were significant positive correlations between semen volume and Na^+ ($r = 0.667$, $p<0.05$), total spermatozoa number and semen volume ($r = 0.977$, $p<0.01$), total spermatozoa number and spermatozoa concentration ($r = 0.652$, $p<0.05$), Na^+ and Cl^- ($r = 0.733$, $p<0.05$), Na^+ and triglyceride ($r = 0.632$, $p<0.05$), K^+ and protein ($r = 0.728$, $p<0.05$), and Ca^{2+} and protein ($r = 0.685$, $p<0.05$). Significant negative correlations were found between Cl^- and glucose ($r = -1.00$, $p<0.05$), and triglyceride and urea ($r = -0.716$, $p<0.05$). Consequently, although there were no definite correlations between biochemical and spermatological parameters, it was observed that a higher Na^+ content has a positive effect on semen volume.

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Introduction

High quality gametes are of great importance in production of viable larvae in fish hatcheries. Semen quality is an important factor that increases the efficiency of artificial fertilization. Techniques for determining semen quality in fish include monitoring semen density, motility, and fertilization success (Tekin et al., 2003a).

Semen consists of seminal plasma and spermatozoa. Seminal plasma contains substances that support sperm cells. Some substances reflect the functioning of the reproductive system and spermatozoa (Akcay et al., 1995; Ciereszko and Dabrowski, 2000). The main role of seminal plasma is to create an optimal environment for spermatozoa storage. Seminal plasma also benefits external fertilization by creating a favorable micro-environment for sperm movement (Billard, 1986). Information on the composition of seminal plasma and other biological fluids can be used to make media for use as a diluent or for gamete storage.

Most experiments on rainbow trout semen have focused on short-term storage or cryopreservation using extenders and cryoprotectants. In rainbow trout, spermatogenesis is a seasonal event (Billard, 1986) and chemical and physical properties of semen may change since all spermatozoa are eliminated by the end of the reproductive season (Lahnsteiner et al., 1993). Better knowledge of semen components is important to understanding events leading to production of good quality gametes and to identifying factors that disturb semen function. The objectives of this study were to examine the biochemical composition of seminal plasma and the physical parameters of rainbow trout semen as well as investigate the influence of biochemical characteristics on the physical parameters at the end of the spawning season.

Materials and Methods

Broodstock care and collection of semen. Ten mature (2–5 years old) male rainbow trout (1.276±4.2 kg, total length 45.25±9.0 cm) were used as semen donors. The broodstock were held in 5 x 5 x 5 m cages under a natural photoperiod regime and fed a commercial trout diet (50% protein) at 1.5–2.0% of their body weight per day. Water temperature varied 10–12°C at the end of the reproductive season (end of March). For semen collection, fish were not fed 48 hours prior to collection, the fish were anesthetized with MS 222 in a 1:10 000 dilution water bath, and the bladders were drained. The semen was collected into 100 ml calibrated glass beakers by gently massaging the abdomens of the fish and only pure samples, uncontaminated by fecal material or urine, were used.

Evaluation of motility, duration of movement, concentration, and pH. To evaluate motility, about 10 µl semen from each fish was placed on a glass microscope slide and 100 µl activation solution (0.3% NaCl) was added. Motility was expressed as the percentage of motile spermatozoa. Observations were made at room temperature (20–23°C) within two hours of semen collection. Duration of spermatozoa movement was estimated using a sensitive chronometer. The concentration of spermatozoa was estimated using the hemocytometric method; semen samples were left on Thoma's hemocytometer undisturbed for a few minutes prior to counting to allow sperm cells to settle. Counts were conducted at 200 magnification and expressed as x 10^9/ml. Semen pH was measured with standard pH electrodes within two hours of sampling.

Determination of seminal plasma composition. Seminal plasma of the semen from each fish was collected after centrifugation of the semen at 3000 rpm for 10 min at room temperature and stored in Eppendorf vials at -20°C until the begining of the analyses. Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, glucose, protein, cholesterol, triglyceride, and urea levels were determined by Abbott-Aeroset autoanalyzer (USA) using original kits.

Statistical analysis. Results are presented as means±SD. Differences between parameters were analyzed by one-way analysis of variance (ANOVA). Significant means were subjected to a multiple comparison test (Duncan) at $\alpha = 0.05$ level. All analyses were carried out using the MINITAB 13.3 for Windows statistical software package.

Results

The physical properties of the semen are presented in Table 1. The composition of the seminal plasma ions and metabolites are shown in Table 2. ANOVA indicated significant differences between individuals in the levels of seminal plasma ions and metabolites ($p<0.05$) but none in the glucose level ($p>0.05$). Correlations between biochemical and physical properties are shown in Table 3.

Discussion

Mean semen volume was similar to results reported by Geffen and Evans (2000), Akcay et al. (2002a), and Tekin et al. (2003b) for rainbow trout *(Oncorhynchus mykiss)* but differed from results reported by McNiven et al. (1993) and Lahnsteiner et al. (1993). The differences may be due to the feeding conditions and regime, water quality, environmental factors, or spawning time. Buyukhatipoglu and Holtz (1984) found that ejaculate volume is significantly higher at the beginning of the spawning season than later.

In our study, the mean spermatozoa motility and duration of spermatozoa movement confirmed results of Munkittrick and Moccia (1987) and Levanduski and Cloud (1988) but differed from those of Akcay et al. (2002a) and Tekin et al. (2003b). Spermatozoa motility varies in vigor and duration not only among males but also within an individual male depending on ripeness (Akcay et al., 2002b). Most studies on fish species have shown that the duration and motility of semen may vary seasonally (Benau and Terner, 1980; Akcay et al., 2004).

The mean spermatozoon concentration in our study agrees with Munkittrick and Moccia (1987), McNiven et al. (1993), and Ciereszko and Dabrowski (1993) but not with Akcay et al. (2002a) and Tekin et al. (2003b). The differences may be due to feeding conditions, age, environmental factors, time of spawning, or dilution ratio. The mean pH generally confirmed Piironen (1985) and Munkittrick and Moccia (1987).

Morisawa (1985) found 75-175 mmol/l Na+, 32-86 mmol/l K+, and 112-183 mmol/l Cl- for teleost fish. Seminal plasma in rainbow trout had a higher Na^+ content than common carp (75 mmol/l; Morisawa et al., 1983) but a lower content than perch (124 mmol/l; Lahnsteiner et al., 1995), catfish (164 mmol/l; Tan-Fermin et al., 1999), and muskellunge (129 mmol/l; Lin et al., 1996). However, the K^+ content was lower than reported for common carp (70 mmol/l) and higher than for Atlantic salmon (28 mmol/l), perch (10 mmol/l), catfish (18 mmol/l), and muskellunge (28 mmol/l). Electrolytes (especially Na^+ and Cl^-) ensure the viability of sperm. The K^+ ion has a role in keeping spermatozoa in the quiescent state (Baynes et al., 1981). Low levels of Na^+ and K^+ ions are associated with low percentages of motile spermatozoa and such semen are considered low quality. However, the high levels and positive correlation determined in our study do not support this situation. Further, Ca^{2+} and Mg^{2+} contribute significantly to the ionic composition of seminal plasma.

The role of protein in fish semen is unknown. White and Macleod (1963) indicated that protein has a protective role. In this

Table 1. Spermatological parameters of rainbow trout *(Oncorhynchus mykiss)* sperm.

	Semen volume (ml)	*Spermatozoa motility (%)*	*Movement duration (s)*	*Spermatozoa concentration (x 10^9/ml)*	*Total no. spermatozoa (x 10^9)*	*pH*
Means±SD	19.9±21.43	60±30.09	174±1.82	1.52±0.70	44.25±64.59	6.7±0.25
Range	7.0–77.0	20–95	45–390	1.204–1.857	8.43–114.73	6.5–7.0

Table 2. Means±standard deviation (n = 3) for seminal plasma ion and metabolite composition of rainbow trout (*O. mykiss*) semen.

Fish no.	*Na^+ (mmol/l)*	*K^+ (mmol/l)*	*Ca^{2+} (mg/dl)*	*Mg^{2+} (mEq/l)*	*Cl^- (mmol/l)*	*Glucose (mg/dl)*	*Protein (g/dl)*	*Cholesterol (mg/dl)*	*Triglyceride (mg/dl)*	*Urea (mg/dl)*
1	48.5±2.50^{b}	20.65±0.75^{a}	5.8±0.30^{e}	4.6±0.10^{d}	52±1.00^{a}	-	0.1±0.00^{a}	1.5±0.50^{abc}	7±0.00^{b}	33.1±3.20^{c}
2	99.5±1.50^{e}	43.8±1.00^{bc}	5.7±0.00^{e}	3.95±0.05^{cd}	115.5±0.50^{de}	-	0.1±0.00^{a}	1±0.00^{ab}	7±0.00^{b}	33.1±3.20^{c}
3	120±2.00^{f}	54±9.00^{e}	5.0±0.20^{d}	2.8±0.10^{b}	141±2.00^{g}	-	0.2±0.00^{b}	2.5±1.50^{bc}	12.5±0.50^{e}	7.49±3.2^{a}
4	13.15±1.85^{a}	39.1±0.30^{b}	2.55±0.05^{a}	3.3±1.00^{bc}	136.5±0.50^{g}	1.5±0.50^{a}	0.1±0.00^{a}	0.5±0.50^{a}	8±2.00^{bc}	4.28±2.14^{a}
5	97.5±4.50^{e}	44.5±1.90^{bc}	4.4±0.40^{c}	3.1±0.00^{bc}	121.5±2.50^{ef}	-	0.1±0.00^{a}	3±0.00^{c}	4.5±0.50^{a}	18.19±3.21^{b}
6	85±20.00^{d}	67.85±6.05^{f}	8.0±0.70^{f}	2.9±1.30^{b}	112.5±10.50^{d}	-	0.4±0.10^{c}	7.5±2.50^{d}	9±0.00^{cd}	18.19±3.21^{b}
7	97±2.00^{e}	46.4±2.90^{cd}	3.7±0.20^{b}	5.9±0.20^{e}	123±3.00^{f}	-	0.15±0.05^{ab}	6.5±0.50^{d}	11.5±0.50^{e}	8.56±2.14^{a}
8	104.5±1.50^{e}	38.4±0.40^{b}	4.1±0.20^{bc}	3.55±0.05^{bc}	128±1.00^{f}	2±1.00^{a}	0.1±0.00^{a}	1±0.00^{ab}	10±0.00^{d}	5.35±1.07^{a}
9	75.5±0.50^{cd}	55.95±0.15^{e}	3.05±0.05^{a}	1.4±0.20^{a}	105±0.00^{c}	0.5±0.50^{a}	0.15±0.05^{ab}	1±0.00^{ab}	3.5±0.50^{a}	140.17±3.21^{e}
10	64.5±1.50^{c}	51.45±1.15^{de}	4.2±0.00^{bc}	3.3±0.00^{bc}	90±2.00^{b}	-	0.1±0.00^{a}	1±0.00^{ab}	7±1.00^{b}	48.15±5.35^{d}
Means ±SD	80.51±31.48	46.21±12.58	4.65±1.57	3.48±1.18	112.5±25.93	1.33±0.76	0.15±0.09	2.55±2.47	8.00±2.84	31.65±40.78

Table 3. Linear correlations between spermatological properties and seminal plasma composition of rainbow trout (*O. mykiss*) semen.

	Volume	*Motility*	*Movement duration*	*Concen-tration*	*Total no.*	*pH*	*Na^+*	*K^+*	*Ca^{2+}*	*Mg^{2+}*	*Cl^-*	*Glucose*	*Protein*	*Choles-terol*	*Tri-glyceride*
Motility	-0.583														
Duration	-0.119	0.001													
Concentration	0.553	-0.259	-0.623												
Total no.	**0.977	-0.555	-0.212	*0.652											
pH	-0.100	-0.461	0.063	-0.406	-0.128										
Na^+	*0.667	-0.381	0.232	0.104	0.582	0.050									
K^+	0.183	-0.447	-0.151	0.126	0.237	0.511	0.480								
Ca^{2+}	-0.383	0.296	0.078	0.007	-0.357	0.075	0.081	0.252							
Mg^{2+}	-0.027	0.193	-0.089	-0.109	-0.121	-0.112	0.107	-0.498	0.068						
Cl^-	0.337	0.104	0.418	-0.077	0.277	-0.313	*0.733	0.036	0.391	0.478					
Glucose	-0.939	0.817	0.831	-0.979	-0.955	-0.189	-0.455	-0.473	0.901	0.766	*-1.000				
Protein	-0.028	-0.039	-0.231	0.105	-0.013	0.456	0.348	*0.728	*0.685	-0.223	0.162	-0.945			
Cholesterol	0.342	-0.459	0.245	0.011	0.343	0.289	0.117	0.014	0.093	-0.238	-0.014	0.696	0.016		
Triglyceride	0.520	-0.013	-0.387	0.438	0.466	-0.264	*0.632	0.083	0.169	0.469	0.589	-0.945	0.290	-0.119	
Urea	-0.627	0.437	0.254	-0.540	-0.528	0.249	0.249	-0.020	0.138	-0.335	-0.359	0.945	-0.039	0.116	*-0.716

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

study, the low protein concentrations indicate a low demand for protein at the end of the spawning season. The positive correlation between the protein level and the K^+ and Ca^{2+} ions can be considered to affect sperm motility. However, because of the low correlation of protein with spermatozoa motility, the role of protein remains unknown. Notable concentrations of urea were found in the semen. Urea is believed to have a relationship with protein metabolism and total protein.

The importance of glucose in fish semen is unclear. The presence of glucose in seminal plasma has been connected to the high energy demand of the testes during spermatogenesis or to lipid synthesis of spermatozoa (Soengas et al., 1993).

Various lipid classes have been found in seminal plasma and their levels vary greatly among fish species, such as 0.007 g/l for Arctic charr (Piironen and Hyvarinen, 1983) and 1.00 g/l for Euroasian perch (Piironen, 1994). Low levels of triglycerides were found in the seminal plasma of cyprinids (Lahnsteiner et al., 1994). According to Piironen (1994), seminal plasma lipids are associated with metabolism in spermatozoa. While cholesterol was found in the seminal plasma of freshwater fish (Billard et al., 1995), there is little information about its role. Lipids and cholesterol might have a protective effect against environmental changes (especially temperature) when semen is released.

Results from our study show that the late spawning season affects semen parameters because there are no definite correlations between spermatological and biochemical parameters. Mature males releasing semen with low motility and low density late in the reproductive season should be culled from the broodstock. Our data can be used to select high quality mature males for fertilizing eggs in a commercial aquaculture operation and, as a result of reducing the number of male broodstock, increase the economic efficiency of the farm. The information on sperm physiology obtained from the present study can lead to more efficient gamete management and increased fry yields, and aid suitability of semen for cryopreservation.

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