# Evaluation of semen quality of endangered Caspian brown trout (*Salmo trutta caspius*) in different times of spermiation during the spawning season

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**ABSTRACT**: Sperm motility and sperm production as well as organic and inorganic components present in the seminal plasma were measured at different time periods of spermiation during the spawning season in order to evaluate the semen quality in Caspian brown trout, *Salmo trutta caspius*. For such evaluation, males were divided into four groups based on the date of spermiation: group A (pre-mature broodstocks), group B and C (mid-mature broodstocks), group D (late-mature broodstocks). Our results showed that the Caspian brown trout produces semen of variable quality depending on the date in the period of spermiation. In this regard, the percentage of motile spermatozoa, duration of motility, sperm density, osmolality and also the concentrations of $Na^+$, $Cl^-$, $K^+$, $Ca^{2+}$, $Mg^{2+}$ and total protein content were significantly higher in mid-mature broodstocks (which matured in the middle of spawning season, i.e. groups B and C) compared to pre-mature (group A) and late-mature (group D) samples. Statistically significant positive correlations were also found between these semen parameters: sperm production vs $Na^+$, $Cl^-$, $K^+$, $Ca^{2+}$, $Mg^{2+}$, total protein, osmolality, pH; percentage of motile spermatozoa vs $Na^+$, $Cl^-$, $K^+$, $Ca^{2+}$, $Mg^{2+}$, total protein, osmolality; duration of motility vs $Na^+$, $Cl^-$, $K^+$, $Ca^{2+}$, $Mg^{2+}$, total protein, pH. The significance of such correlations is interpreted in terms of physiological implications and is discussed in relation to semen quality.

**Keywords**: sperm motility; spermiation; spawning season; Caspian brown trout

The Caspian brown trout, *Salmo trutta caspius*, is a critically endangered anadromous species that has been considered for a biological conservation program in the southern part of the Caspian Sea (Kiabi et al., 1999). In recent years, because of a dramatic decline in broodstock capture and subsequently of insufficient availability of sexual gametes (sperm and ova), severe restrictions were imposed on the propagation of this species by means of artificial fertilization. Thus, many studies have been carried out to eliminate this problem by using technologies such as cryopreservation of Caspian brown trout semen in order to solve the problem of permanent

access to spermatozoa (Sarvi et al., 2006). For such purpose, the selection of the best quality semen is the most critical point to increase the efficiency of artificial fertilization after sperm thawing. A survey of literature shows that the main parameters which determine the fish semen quality are as follows:

(1) Composition of seminal plasma: seminal plasma has a unique composition regarding the presence of organic and inorganic components which support the viability of spermatozoa as well as substances reflecting the function of the reproductive system like spermatozoa (Piironen and Hyvarinen, 1983; Lahnsteiner et al., 1993

(a,b); Ciereszko et al., 2000). In this regard, the inorganic components present in seminal plasma (potassium, sodium, magnesium, calcium, chloride, pH and osmolality) provide isotonic conditions for spermatozoa and inhibit sperm motility (Morisawa et al., 1983). The organic components including glucose and triglycerides act as the main energy resources for ATP generation (Billard and Cosson, 1990) and others such as proteins prolong the viability of spermatozoa (Lahnesteiner et al., 2004).

(2) Sperm motility and sperm density: these parameters determine the fertilization capability of spermatozoa and are often used to estimate the semen quality (Suquet et al., 1982; Billard et al., 1993; Linhart et al., 1994; Krol et al., 2006).

On the basis of the above-mentioned criteria, the main semen quality parameters in the present study were measured in Caspian brown trout during the spawning season at different time periods of spermiation: these parameters included organic components (triglycerides, glucose and total protein), inorganic components ($Na^+$, $Cl^-$, $K^+$, $Ca^{2+}$, $Mg^{2+}$, pH, osmolality), sperm density and sperm motility. The aims of the present investigation are:

(a) to identify parameters correlated with sperm motility which might be used as biomarkers for semen evaluation and selection of the highest quality semen before insemination;

(b) to determine the appropriate time period for male stripping by monitoring the semen quality in different time periods of spermiation during the spawning season.

## MATERIAL AND METHODS

The experiment was carried out at the Kalardasht Salmonids Reproduction Centre (KSRC), Iran, during the spawning season of Caspian brown trout. Altogether, 42 males (TL: 57.9 ± 2.3 cm, TW: 1 580.2 ± 160 g) were captured from the Sardabrood River during their upstream reproductive migration, and then transferred to a broodstock pond (3 m × 9 m × 1.5 m with water flow about 150 l/min) at the KSRC. Males were checked up from 5 November at 6–8 day intervals by hand stripping and divided into four groups based on the date of spermiation (Table 1) (groups were identified by distinct tags): group A, 18 November ($n$ = 12): these broodstocks matured in the early phase of spawning season and were named as pre-mature (PM) broodstocks. Group B, 2 December ($n$ = 11) and group C, 16 December ($n$ = 10): these broodstocks matured in the middle of spawning season and were named as mid-mature (MM) broodstocks. Group D, 29 December ($n$ = 9): these broodstocks

Table 1. The organic and inorganic components of seminal plasma, sperm density and duration of motility pooled over all groups of Caspian brown trout in different times of spermiation during the spawning season

| Semen parameters | Minimum | Maximum | Mean | SD |
|---|---|---|---|---|
| Sperm density (×10⁹cells/ml semen) | 0.8 | 5.3 | 3.14 | 1.24 |
| Duration of motility (s) | 24 | 48 | 39 | 7.2 |
| $Na^+$ (mM/l) | 117 | 154 | 135.4 | 10.6 |
| $Cl^-$ (mM/l) | 91.9 | 160.1 | 139 | 15.9 |
| $K^+$ (mM/l) | 20.1 | 46.7 | 31.6 | 7.1 |
| $Ca^{2+}$ (mM/l) | 0.8 | 2.1 | 1.5 | 0.4 |
| $Mg^{2+}$ (mM/l) | 0.7 | 2.1 | 1.2 | 0.2 |
| Triglycerides (mM/l) | 0.2 | 0.5 | 0.4 | 0.1 |
| Glucose (mM/l) | 1.1 | 4.3 | 1.9 | 0.1 |
| Total protein (mg/ml) | 0.1 | 0.9 | 0.4 | 0.2 |
| Osmolality (mOsmol/kg) | 162 | 240 | 198.4 | 18.3 |
| pH | 7.3 | 8.2 | 7.9 | 0.2 |

SD = standard deviation

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matured at the end of spawning season and were named as late-mature (LM) broodstocks.

Before stripping, the fish were anaesthetized using 100 ppm of MS222 (tricaine methane sulphonate), then the semen collection was carried out by massage from the anterior portion of the belly (testis region) towards the genital papilla. Special care was taken to avoid the contamination of semen by water, mucus, blood cells, faeces or urine. Immediately after semen collection, the osmotic pressure and pH of semen were measured with an osmometer (Melting Point Osmometer Nr 961003, Roebling Company, Berlin, Germany) and a pH meter with a semi-microelectrode using an external reference (3.0 mmol/l KCl), respectively. Semen samples were stored for less than one hour at 5°C until motility and the sperm density analysis was carried out.

## Sperm motility

Sperm motility of each sample was evaluated within 40 min after semen collection. A two- step dilution was used for motility activation according to the method suggested by Billard and Cosson (1992) for salmonid fish. Firstly, the semen was pre-diluted to a saline solution (composed of 4.68 g NaCl, 2.98 g KCl, 0.11 g $CaCl_2$, 3.15 g Tris-HCl per one litre of distilled water (final pH = 9) at a ratio of 1/100 and secondly, the prediluted semen was subjected to a second dilution in a physiological serum (0.7% of NaCl) at a ratio of 1/20 and immediately 1 µl of solution was placed on the microscope stage and motility was analyzed by a semi-quantitative method (Rurangwa et al., 2004). In this regard, the motility was recorded on a videotape in the light microscopic field from about 5–7 s after the onset of motility until at least 90% of cells were immotile. Then, the video recorded images were transferred to a computer and analyzed using jetAudio software by three independent observers. All observations on the computer were investigated using the slow motion facility and only forward-moving spermatozoa were classified as motile, while sperm cells simply vibrating or turning on their axes were considered as immotile (Aas et al., 1991).

## Sperm density

Two different methods were used to determine the sperm density:

## Spermatocrit method

The spermatocrit is defined as the ratio of white packed material volume to the total volume of semen × 100 (Rurangwa et al., 2004). Microhaematocrit capillary tubes (75 mm in length and 1.1–1.2 mm in diameter) were filled with semen and one end of each tube was sealed with clay. The capillary tubes were centrifuged at 5 000 rpm for 10 min in a D-78532 centrifuge (Tuttlingen, Zentrifugen, Germany) and the pellet volume was referred to the total volume introduced in the capillary.

## Spermatozoa counting method

Semen was diluted 1 000 times by pipetting 10 µl semen in 990 µl of 0.7% NaCl (Ciereszko and Dabrowski, 1993). A haemocytometer counting chamber was used to determine the spermatozoa density. A droplet of the diluted milt was placed on a haemocytometer slide (depth 0.1 mm) with a coverslip and counted using light microscopy. After 3–5 min (to allow sperm sedimentation), the number of spermatozoa was counted in 16 individual cells, then calculated according to Caille et al. (2006).

## Determination of organic and inorganic components of seminal plasma

To analyze the chemical components of seminal plasma, the semen was separated from the seminal plasma by centrifugation (Heraeus, Sepatech, Labofuge 200, Germany, 5 000 rpm for 10 min). Magnesium, chloride and calcium were measured by a colorimetric method using an Auto-analyser Technican (RA 1000, Technicon-Swords, Dublin, Ireland). Potassium and sodium were determined using a flamephotometer (CORNING 480, Corning, Medfield, MA, USA). Glucose, triglycerides and total protein of seminal plasma were measured spectrophotometrically (Cintra 40 UV-Visible Spectrometer, GBC) (standard analysis kits from Ziestchem, Tehran, Iran).

## Statistical analysis

The SPSS software was used to analyze data. All parameters were expressed as arithmetical means and standard deviations; one-way ANOVA was em-

Table 2. The values of sperm motility (percentage and duration), sperm density, spermatocrit and the organic and inorganic components of seminal plasma of Caspian brown trout in different times of spermiation during the spawning season

| Traits | Groups of broodstocks | | | |
|---|---|---|---|---|
| | A | B | C | D |
| Motility(%) | $54.2 \pm 6.33^a$ | $66.8 \pm 4.1^b$ | $70 \pm 4.2^b$ | $58.8 \pm 8.6^a$ |
| Duration of motility | $28.4 \pm 2.6^a$ | $44 \pm 1.75^b$ | $42.9 \pm 2.6^b$ | $42.9 \pm 2.71^b$ |
| Sperm density | $1.9 \pm 0.7^a$ | $4.4 \pm 0.6^b$ | $4 \pm 0.2^b$ | $2.2 \pm 0.5^a$ |
| Spermatocrit | $28.8 \pm 5.1^a$ | $45 \pm 5.7^b$ | $44.4 \pm 4.6^b$ | $45.6 \pm 4.2^b$ |
| $Na^+$ | $128.9 \pm 8.7^a$ | $143.4 \pm 9.2^b$ | $138.8 \pm 11.2^b$ | $130.8 \pm 5.9^a$ |
| $Cl^-$ | $132.3 \pm 17.9^a$ | $148.4 \pm 8.6^b$ | $145.5 \pm 6.8^{bc}$ | $129.4 \pm 19^{ac}$ |
| $K^+$ | $28.6 \pm 5.1^a$ | $38.5 \pm 7.3^b$ | $33.9 \pm 3.4^c$ | $25.4 \pm 3.6^a$ |
| $Ca^{2+}$ | $1.1 \pm 0.2^a$ | $1.9 \pm 0.2^b$ | $1.9 \pm 0.3^b$ | $1.3 \pm 0.3^c$ |
| $Mg^{2+}$ | $1 \pm 0.1^a$ | $1.3 \pm 0.2^b$ | $1.5 \pm 0.2^b$ | $1.2 \pm 0.1^c$ |
| Triglycerides | $0.4 \pm 0.2^a$ | $0.3 \pm 0.1^a$ | $0.4 \pm 0.1^a$ | $0.3 \pm 0.2^a$ |
| Glucose | $2 \pm 0.1^a$ | $1.9 \pm 0.9^a$ | $1.8 \pm 0.9^a$ | $1.9 \pm 0.9^a$ |
| Total protein | $0.2 \pm 0.1^a$ | $0.6 \pm 0.1^b$ | $0.5 \pm 0.1^c$ | $0.3 \pm 0.1^a$ |
| Osmolality | $188.6 \pm 18.3^a$ | $209 \pm 14.6^b$ | $205.6 \pm 20.8^b$ | $190.6 \pm 8.2^a$ |
| pH | $7.65 \pm 0.23^a$ | $8.03 \pm 0.13^b$ | $8.01 \pm 0.14^b$ | $7.98 \pm 0.10^b$ |

*values are means ± SD; means with same superscripts are not significantly different ($P > 0.05$); $Na^+$, $K^+$, $Cl^-$, $Ca^{2+}$, $Mg^{2+}$; glucose, triglycerides (mM/l); total protein (mg/ml); osmolality (mOsmol/kg); sperm density ($\times 10^9$ cells/ml semen); spermatocrit (%); duration of motility (second)

ployed to analyze data. Then, means were compared by Tukey's test. Data measured in percentage scale (spermatocrit and percentage of motile spermatozoa) were converted by angular transformation (arcsin $\sqrt{p}$) prior to analysis. The relationships between parameters of the seminal plasma, sperm motility (percentage of motile spermatozoa and duration of motility) and sperm density were tested using the bivariate Pearson correlation coefficients. Linear and non-linear regression models were investigated using regression fits. The total duration of sperm motility and the percentage of motile spermatozoa were used as dependent variables and seminal plasma parameters as independent variables, in contrast, the sperm density was used as an independent variable and seminal plasma parameters as dependent variables.

## RESULTS

### Sperm production and sperm motility

Duration of motility, percentage of motile spermatozoa, sperm density and organic and inorganic components pooled over all groups of Caspian brown trout are shown in Table 1. The means of spermatocrit in mid-mature (groups B and C) and late-mature broodstocks (group D) were significantly higher than in pre-mature (group A) broodstocks (Table 2, $P < 0.05$). Also, fish in groups B and C had a significantly higher sperm density and percentage of motile spermatozoa than fish in groups A and D (Table 2, $P < 0.05$). Furthermore, fish in groups B, C and D showed longer duration of motility than group A (Table 2, $P < 0.05$).

The concentrations of $Na^+$, $K^+$, $Ca^{2+}$, $Mg^{2+}$, $Cl^-$, osmolality and total protein were significantly higher in groups B and C than in the other groups (Table 2, $P < 0.05$). There were no significant differences in triglyceride and glucose concentrations among all groups (Table 2, $P > 0.05$). The semen pH value was lower in group A compared with pH values obtained in the mid-mature (groups B and C) and late-mature (group D) broodstocks (Table 2, $P < 0.05$).

Significant relationships were found between sperm density and spermatocrit, $Ca^{2+}$, $Mg^{2+}$, $K^+$, $Cl^-$,

Table 3. Correlations between the organic and inorganic components of seminal plasma, sperm density, percentage of motile spermatozoa and duration of motility in Caspian brown trout ($n$ = 42 semen samples)

|  | Sperm density | Motility (%) | Duration of motility (s) |
|---|---|---|---|
| Na$^+$ | 0.545** | 0.540** | 0.326** |
| Cl$^-$ | 0.511** | 0.408** |  |
| K$^+$ | 0.762** | 0.590** | 0.331* |
| Ca$^{2+}$ | 0.533** | 0.572** | 0.629** |
| Mg$^{2+}$ | 0.703** | 0.562** | 0.539** |
| Glucose | – | – | – |
| Triglycerides | – | – | – |
| Total protein | 0.847** | 0.515** | 0.523** |
| Osmolality | 0.476** | 0.399** | – |
| pH | 0.550** | – | 0.703** |

*statistically significant relationships are indicated as follows: **$P < 0.01$, *$P < 0.05$ and non-significant as; data: bivariate coefficient

Na$^+$ and total protein, osmolality and pH ($P < 0.01$); between the percentage of motile spermatozoa and Ca$^{2+}$, Mg$^{2+}$, K$^+$, Cl$^-$, Na$^+$, total protein and osmolality ($P < 0.01$), also between the duration of motility and Ca$^{2+}$, Mg$^{2+}$, K$^+$, Na$^+$, total protein and pH ($P < 0.01$) (Table 3). The highest correlations were observed between sperm density and spermatocrit ($r^2$ = 0.96, $P < 0.01$, Figure 1), percentage of motility and K$^+$ ($r^2$ = 0.65, $P < 0.01$, Figure 2) and between the duration of motility and pH ($r^2$ = 0.50, $P < 0.01$, Figure 3). Significant correlations were also found between seminal plasma parameters (including Ca$^{2+}$, Mg$^{2+}$, K$^+$, Cl$^-$, Na$^+$, total protein, pH and osmolality)

that are shown in Table 4. In our study, there was no relationship between semen parameters and the weight of broodstocks (as a biometrical character) ($P > 0.05$).

## DISCUSSION

### Sperm production

The present study showed that the Caspian trout produces semen with low sperm density (0.8 – 5.3 × 10$^9$, 3.2 ± 1.31 × 10$^9$, pooled over all groups) com-
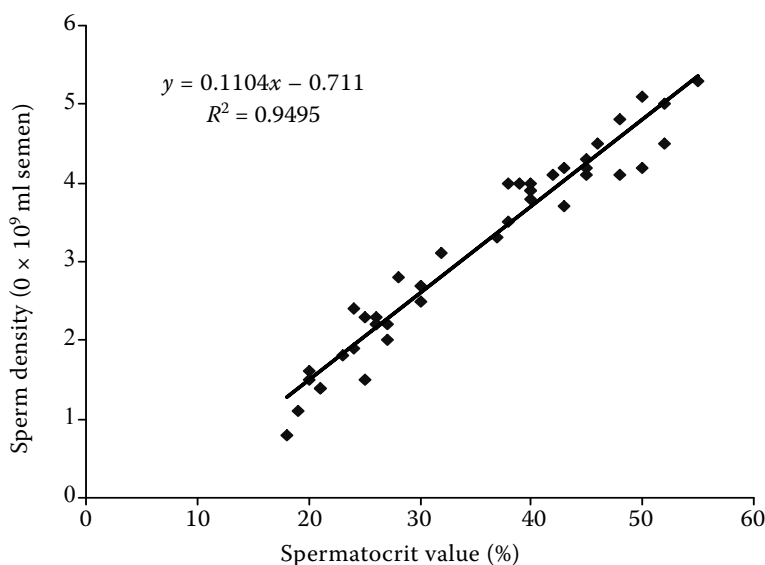


$y = 0.1104x - 0.711$
$R^2 = 0.9495$

Figure 1. A relationship between spermatocrit and sperm density in Caspian brown trout ($n$ = 42 semen samples; independent variable: spermatocrit, dependent variable: sperm density)
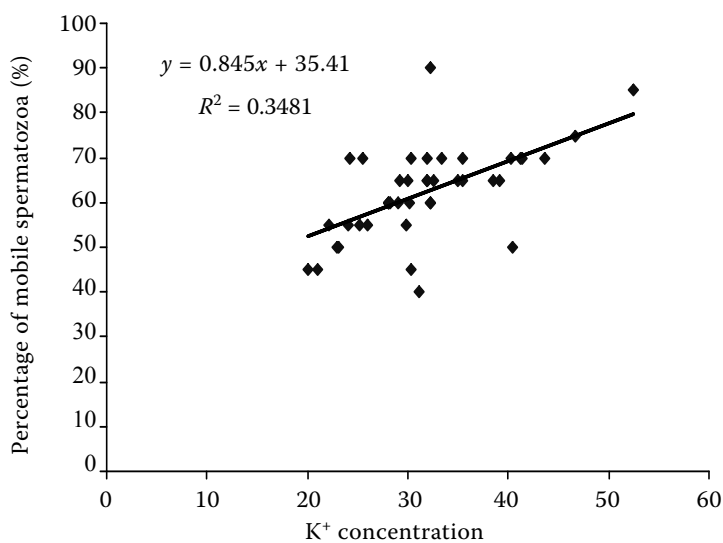
Figure 2. A relationship between K⁺ concentration and percentage of motility in Caspian brown trout (*n* = 42 semen samples, independent variable: K⁺ concentration, dependent variable: percentage of motility)
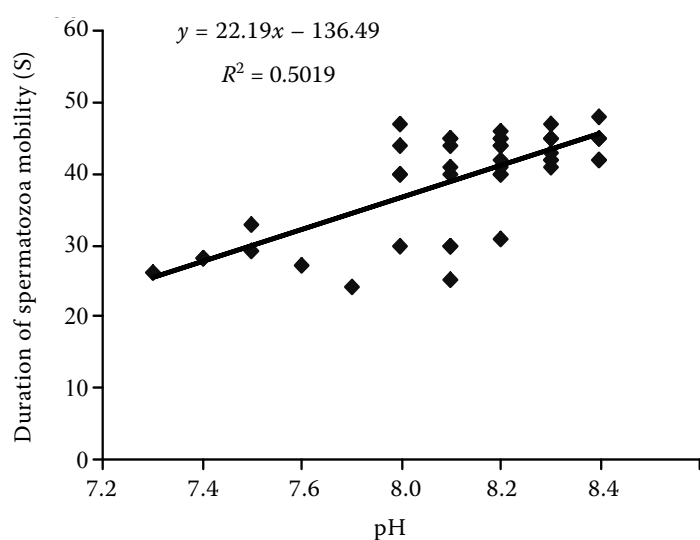


Figure 3. A relationship between pH and duration of motility in Caspian brown trout (*n* = 42 samples, independent variable: pH, dependent variable: duration of motility)

Table 4. Correlations between organic and inorganic components of seminal plasma of Caspian brown trout (*n* = 42 semen samples)

| | $Na^+$ | $Cl^-$ | $K^+$ | $Ca^{2+}$ | $Mg^{2+}$ | Glucose | Trigly-cerides | Total protein | Osmo-lality | pH |
|---|---|---|---|---|---|---|---|---|---|---|
| $Na^+$ | – | 0.679 | 0.488 | 0.462 | 0.311 | – | – | 0.540 | 0.852 | – |
| $Cl^-$ | ** | – | 0.344 | 0.541 | ₅ | – | – | 0.493 | 0.736 | – |
| $K^+$ | ** | * | – | 0.504 | 0.413 | – | – | 0.666 | 0.359 | 0.323 |
| $Ca^{2+}$ | ** | ** | ** | – | 0.514 | – | – | 0.738 | 0.398 | 0.493 |
| $Mg^{2+}$ | ** | | ** | ** | – | – | – | 0.540 | 0.351 | 0.314 |
| Glucose | – | – | – | – | – | – | – | – | – | – |
| Triglycerides | – | – | – | – | – | – | – | – | – | – |
| Total protein | ** | ** | ** | ** | * | – | – | – | 0.399 | 0.405 |
| Osmolality | ** | ** | * | ** | ** | – | – | ** | – | – |
| pH | – | | * | ** | | – | – | ** | | – |

*statistically significant relationships are indicated as follows: **$P$ < 0.01, *$P$ < 0.05 and non-significant as; data: bivariate coefficient

pared to other salmonid fish. In this regard, for rainbow trout, *Oncorhynchus mykiss* sperm densities of $11.8 \pm 6.19 \times 10^9$ (Ciereszko and Dabrowski, 1993), $8.9 \pm 3.8 \times 10^9$ (Geffen and Evans, 2000), for Atlantic salmon, *Salmo salar*, $3.5 – 17.9 \times 10^9$ (Aas et al., 1991), $12 – 30 \times 10^9$ (Truscott and Idler, 1969) were reported. Different values of sperm production were also recorded for each group during the spawning season. Several studies have mentioned that the differences in sperm production could be related to many factors including the age and weight of the male (Suquet et al., 1994, 1998), ecology and spawning behaviour of broodstock (Piironen and Hyvarinen, 1983) and sampling period and method (Suquet et al., 1994). In present study, there was no significant relationship between broodstock weight and sperm production. Variation in broodstock weight was comparatively low for all groups. Thus it seems probable that the influence of broodstock weight on sperm production is reflected in high weight variations. Furthermore, changes in the sperm production pattern in different times of spermiation could be related to the influence of sampling period on sperm production during the spawning season. In our results, significant positive relationships were found between sperm density and the concentrations of $Na^+$, $Cl^-$, $K^+$, $Ca^{2+}$, $Mg^{2+}$ and total protein in the seminal plasma. In many previous researches, similar relationships were observed between sperm density vs $Na^+$, $K^+$, $Ca^{2+}$, $Mg^{2+}$ in landlock salmon, *Salmo salar M. Sebago Girard* (Piirenen, 1985), sperm density vs total protein in Siberian sturgeon, *Acipenser baeri* (Piros et al., 2002) and in a previous study in Caspian brown trout, sperm density vs $Ca^{2+}$, $Mg^{2+}$ and total protein (Hatef et al., 2007). Similarly, the close correlations between the concentration of $Na^+$, $Cl^-$, $K^+$, $Ca^{2+}$, $Mg^{2+}$ and sperm density reveal a regulatory role of these minerals during spermiation of Caspian brown trout (Piirenen, 1985; Hatef et al., 2007). Lahnsteiner et al. (2004) reported that some proteins of seminal plasma were shown to have a key role in the motility of sperm cells. It is possible that in dense semen, higher concentrations of these types of proteins are required for the stimulation of sperm motility than in semen with low sperm density (Hatef et al., 2007). Similarly like in some teleost fish (Takashima et al., 1984; Aas et al., 1991; Ciereszko and Dabrowski, 1993; Hatef et al., 2007), a positive relationship between sperm density and spermatocrit was found in Caspian brown trout. Thus, such a relationship suggests that the evalu-ation of sperm quantity by spermatocrit determination is better than the spermatozoa counting method from facility and time saving aspects.

## Organic and inorganic components of seminal plasma

The mineral composition of the seminal plasma pooled over all groups (A, B, C, D) is in the range of salmonid fish reported by Billard et al. (1995): $Na^+$: 103–140mM/l, $K^+$: 20–66mM/l, $Ca^{2+}$: 0.3–2.6mM/l and $Mg^{2+}$: 0.8–3.6mM/l but the concentration of $K^+$: $20.1 – 46.7$, $31.6 \pm 7.1$mM/l was higher than in rainbow trout (Lahnsteiner et al., 1998; Glogowski et al., 2000) and Atlantic salmon (Hwang and Idler, 1969). Generally, interactions of ions present in the seminal plasma with the sperm membrane influence the membrane potential (Ciereszko et al., 2000) and represent a mechanism of inhibition of spermatozoa in the seminal plasma or sperm duct (Boitano and Omoto, 1991), allowing to control the initiation of sperm motility after release to the surrounding medium (Krasznai et al., 2000). The total protein concentration in the present study ($0.1–0.9$, $0.4 \pm 0.2$ mg/ml) was lower than that reported for salmonids such as rainbow trout: $1.74 \pm 0.79$ mg/ml (Loir et al., 1990), $1.34 \pm 0.67$ mg per ml (Ciereszko and Dabrowski, 1993) and $1.47 \pm 0.84$ mg/ml (Lahnsteiner et al., 1998). In addition, the protein content is significantly different from that of non-salmonid fish (Piironen and Hyvarinen, 1983; Lahnsteiner et al., 1995; Lahnsteiner et al., 1996). Mostly, the seminal plasma of teleost fish is characterized by a very low concentration of proteins in comparison with mammalian semen and does not exceed 2 g/l (Krol et al., 2006). This problem was proved for Caspian brown trout with the mean of 0.4 g/l total protein pooled over all groups during the spawning season. Fish have no accessory glands which contribute to the production of most seminal plasma proteins in mammals (Billard, 1986), thus the low concentrations of seminal plasma proteins can be related to the absence of accessory glands in fish. The glucose and triglyceride concentrations in the present study (glucose: $1.1–4.3$, $1.9 \pm 0.9$mM/l, triglycerides: $0.2–0.5$, $0.4 \pm 0.1$mM/l, pooled over all groups) were higher than those reported for salmonids such as Atlantic salmon (glucose: $0.45 \pm 0.15$mM/l) (Aas et al., 1991), rainbow trout (triglycerides: $0.18 \pm 0.12$mM/l) (Lahnsteiner et al., 1998) as well as for

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non-salmonid fish such as Eurasian perch, *Perca fluviatilis* (glucose: 0.063 ± 0.019mM/l, triglycerides: 0.072 ± 0.1mM/l) (Lahnesteiner et al., 1995), burbot, *Lota lota* (glucose: 0.00762 ± 0.00764mM/l, triglycerides: 0.078 ± 0.027mM/l) (Lahnesteiner et al., 1997). Commonly, monosaccharides and lipids such as glucose and triglycerides serve as energy sources for sperm motility in fish (Stoss, 1983; Lahnesteiner et al., 1993b), thus different concentrations of glucose and triglycerides in fish seminal plasma could be related to differences in spermatozoa energy metabolism among fish species. High levels of glucose concentrations in the Caspian brown trout seminal plasma may be related to stress conditions (confinement or holding, handling, etc.) in hatchery and subsequently, an increase in the glucose concentration of body fluids (such as seminal and blood plasma) follows because of the constant activity of glycolysis pathway in liver in response to stress conditions (Portz et al., 2006). In general, differences in the organic and inorganic composition of seminal plasma in fish probably represent species-specific characteristics among fish species (Ciereszko et al., 2000; Alavi et al., 2004; Hatef et al., 2007) that should be considered when procedures for artificial insemination or storage of sperm (both short-term storage and cryopreservation) are envisaged (Billard et al., 1995; Rurangwa et al., 2004; Alavi and Cosson, 2006). In the present study, inorganic and some organic components of seminal plasma showed different values in different times of spermiation. In fish, the formation of the seminal plasma (inorganic as well as organic components) is a secretion process of the spermatic duct epithelium (Marshall, 1986; Marshall et al., 1989; Lahnsteiner et al., 1993a, 1994), therefore the differences in the composition of seminal plasma could be related to a change in secretory activity in the Caspian brown trout spermatic duct in different times of spermiation during the spawning season. The semen pH in pre-mature broodstocks of Caspian brown trout was lower than in the other groups. The previous studies in salmonid fish suggested that the fluctuations in semen pH are related to the secretion of bicarbonate into seminal plasma by the spermatic duct epithelium in the time of sperm final maturation (Morisawa and Morisawa, 1988). Thus, the low semen pH in pre-mature broodstocks may be related to a deficiency in bicarbonate secretion by the spermatic duct epithelium in the early spawning season (Lahnsteiner et al., 1996). The semen osmolality in

pre-mature and late-mature broodstocks was lower than in the broodstocks that matured in the middle of spawning season. The relationships between osmolality and the concentrations of $Na^+$, $Cl^-$, $K^+$, $Ca^{2+}$, $Mg^{2+}$, and total protein were also observed. Several studies showed significant correlations between the mineral content of seminal plasma and osmolality (Alavi and Cosson, 2006). Thus, in the present study, the differences in semen osmolality can depend on changes in the mineral content and likely in some organic components of the seminal plasma in different times of spermiation during the spawning season. With regard to this fact, $Na^+$ and $Cl^-$ are probably the main electrolytes that play a major role in maintaining the osmolality of seminal plasma (Morisawa et al., 1979) as the high correlation was recorded between osmolality and the concentrations of $Na^+$ and $Cl^-$ in Caspian brown trout. In addition, other factors including semen contamination by urine during stripping (Suquet et al., 1994) and hydration of semen during the spermiation period (Morisawa et al., 1979) may affect the fish semen osmolality.

## Sperm motility

In this study, the percentage of motile spermatozoa and the duration of motility showed specific change patterns on the basis of spermiation time during the spawning season. On the other hand, there were significant positive relationships between the percentage of motile spermatozoa and $Na^+$, $Cl^-$, $K^+$, $Ca^{2+}$, $Mg^{2+}$, total protein and osmolality, as well as between the duration of motility and $Na^+$, $K^+$, $Ca^{2+}$, $Mg^{2+}$, total protein and pH. In agreement with our results, significant relationships were found between the percentage of motile spermatozoa and $Na^+$, $K^+$, pH and osmolality in rainbow trout (Lahnsteiner et al., 1998), percentage of motile spermatozoa and pH in Chinook salmon, *Oncorhynchus tshawytscha* (Ingermann et al., 2002), also in non-salmonid fish: percentage of motile spermatozoa vs $Na^+$, $Mg^{2+}$, $Cl^-$, the duration of motility vs $Na^+$ in Persian sturgeon, *Acipenser persicus* (Alavi et al., 2004), percentage of motile spermatozoa vs $Na^+$, $K^+$, total protein, pH, osmolality in *Alburnus alburnus* (Lahnsteiner et al., 1996), although the negative relationships were recorded between the percentage of motility and $Ca^{2+}$, $Mg^{2+}$, $Na^+$, $K^+$ in European eel, *Anguila anguila* (Perez et al., 2003). The existence of such

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relationships in our study and in other studies suggests that the quantity of inorganic and some organic components in the fish seminal plasma affects the motility potential of spermatozoa before releasing to the environmental media (Alavi and Cosson, 2006). This effect can be different depending on the species. With regard to this fact, the highly significant correlations between the percentage of motile spermatozoa and. $K^+$ and between the duration of motility and pH suggest that these parameters may be the most important seminal plasma characteristics influencing the sperm activation and the durability of spermatozoa motility in Caspian brown trout, respectively. According to previous studies, during the passage of spermatozoa from the testis to the spermatic duct an increase in external pH may be responsible for the acquisition of motility in some salmonid fish (Morisawa and Morisawa, 1986, 1988; Billard et al., 1995) and therefore the seminal fluid pH may also effect the final maturation of spermatozoa (Lahnesteiner et al., 1998). Thus, the significant correlation between the duration of motility and semen pH in Caspian brown trout may be related to this problem. Also, the significant relationship between the percentage of motility and total protein in seminal plasma could be related to the key role of some proteins in the motility of sperm cells (Lahnestiner et al., 2004) or their influences on motility by buffering the seminal plasma, as proteins also reveal a positive correlation with pH (Lahnestiner et al., 1996). As mentioned above, the lipids and monosaccharides such as triglycerides and glucose serve as energy resources for energy metabolism of spermatozoa (Stoss, 1983; Lahnesteiner et al., 1993b). Low triglyceride and glucose levels could therefore be indicative of inadequate energy resources, reduced motility rate and fertilization capacity (Lahnestiner et al., 1998). On the other hand, in recent study, there were no relationships between the sperm motility and concentrations of triglycerides and glucose. Therefore, this problem could be due to the existence of a threshold for these components in relation to sperm motility, so that probably the influence of these parameters on sperm motility is cleared in very low concentrations.

## CONCLUSION

In Caspian brown trout, the determination of semen quality parameters reveals that the broodstocks produce the semen of different quality depending on the date of spermiation during the spawning season. In this regard, the measurement of organic and inorganic components in seminal plasma, motility of spermatozoa, sperm production and determination of physiological relationships between these parameters document that the broodstocks that matured in the middle of spawning season produce the semen of better quality than the others in the early and late spawning season. Therefore, the semen properties of mid-mature broodstocks can be used as quality biomarkers for artificial fertilization purposes such as evaluation of semen quality before insemination and formulation of extender solution for cryopreservation and short-term storage of sperm. The proposed solution should contain: $Na^+$ (125 – 162, 141.2 ± 10.2mM/l), $Cl^-$ (131 – 160.1, 147 ± 7.7mM/l), $K^+$ (29.2 – 52.4, 36.3 ± 6.1mM/l), $Ca^{2+}$ (1.3 – 2.1, 1.9 ± 0.2mM/l), $Mg^{2+}$ (1.1 – 1.7, 1.4 ± 0.2mM/l), triglycerides (0.24 – 0.45, 0.35 ± 0.04mM/l), glucose (1.19 to 4.31, 1.8 ± 0.9mM/l), total protein (0.4 – 0.9, 0.5 ± 0.7mM/l), osmolality (171 – 240, 207.3 ± 17.4 mOsmol/kg), pH (7.8 – 8.2, 8 ± 0.1). The explanation of relationships between sperm motility and other components of semen might provide further knowledge of the metabolic pathways important for motility and serve as a basis for further studies evaluating the fertilization capacity of spermatozoa based on biochemical criteria.

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