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SHORT COMMUNICATION

# Protein concentration and enzyme activities of fresh and frozen–thawed Persian sturgeon, *Acipenser persicus* (Borodin, 1897) semen

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**Abstract** The membrane integrity of fish spermatozoa can be compromised after exposure to a freeze–thaw cycle, resulting in leakage of proteins in the seminal fluid. In the present work, the total protein concentration (TP) and enzyme activities of acid phosphatase (AcP), lactate dehydrogenase (LDH), arylsulfatase (AS), *β-N*-acetylglucosaminidase (*β-N*-AGase) were measured in seminal plasma of fresh and freeze–thawed semen of Persian sturgeon (*Acipenser persicus* Borodin). The enzyme activity of fresh and frozen–thawed seminal plasma was as: AcP ($0.59 \pm 0.035$ and $9.89 \pm 0.81$), LDH ($484.4 \pm 27.3$ and $6{,}262.7 \pm 263.2$), AS ($169.1 \pm 44.4$ and $699.6 \pm 25.1$), *β-N*-AGase ($36.31 \pm 2.25$ and $70.7 \pm 2.24$), respectively, in fresh and freeze–thawed sperm. The TP semen content in fresh semen was $0.28 \pm 0.031$ and in frozen–thawed was $1.68 \pm 0.1$. The results indicate that TP concentration and activities of AcP, LDH, AS, and *β-N*-AGase were significantly higher in frozen–thawed seminal plasma. For this reason, these molecules may be used as markers for estimation of membrane damage of spermatozoa exposed to freeze–thaw cycle.

**Keywords** Protein content · Enzyme activities · Seminal plasma · Frozen–thaw cycle · *A. persicus*

## Background

Sturgeons are considered to be ‘‘living fossils’’ (Bemis et al. 1997). Their evolutionary history dates back to the early Jurassic period (approximately 100–200 million years ago). The Caspian Sea is home to the four commercial species of sturgeon, including the Persian sturgeon, *Acipenser persicus* Borodin (Khodorevskaya et al. 1997). However, their populations are declining due to excessive fishing for meat and caviar production, habitat destruction, and water pollution (Billard and Lecointre 2001). Thus, there has been an increased demand for information about all aspects of sturgeon reproduction (Billard and Lecointre 2001; Baker et al. 2005; Aramli et al. 2013a).

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Studies of the usefulness of biochemical indicators as estimators of semen quality generally focus on parameters measured from plasma from fresh semen and supernatants of cryopreserved semen. Enzyme activities are measured in sperm extracts or in the supernatants after long- and short-term storage (Ciereszko 2008). Assessment of the composition and functions of proteins and enzymes provides insights into the spermatozoa motility and fertilizing abilities that remain after processing. Identifying optimum processing techniques and storage conditions will improve artificial reproduction programs and germplasm resource conservation procedures.

Seminal plasma proteins help protect spermatozoa during storage in the reproductive organ, whereas all semen proteins contribute to the swimming and fertilizing abilities of sperm (Li et al. 2008). Compared with what is known about mammalian semen, the enzymology of fish gametes is poorly understood. However, activities of aspartate aminotransferase, lactate dehydrogenase (LDH), acid phosphatase (AcP), *β-N*-acetyl-glucosaminidase (*β-N*-AGase), anti-proteinase, and arylsulfatase (AS) in fish semen have been studied previously (Schemehl et al. 1987; McNiven et al. 1992; Ciereszko and Dabrowski 1994; Piros et al. 2002; Sarosiek et al. 2004, 2006; Aramli et al. 2013b).

In contrast to gametes from teleost fish, sturgeon sperm are characterized by the presence of an acrosome (Jamieson 1991) and oocytes contain multiple micropyles (Cherr and Clark 1985). Previous studies of the sturgeon acrosome focused on acrosin-like activity of the sperm (Ciereszko and Dabrowski 1994; Ciereszko et al. 1996, 2000) and activities of AS and *β-N*-AGase (Piros et al. 2002). The acrosomal enzymes of sturgeon may be important in certain situations. For example, prolonged contact of oocytes with water may lead to micropyle obstruction by the jelly coat layer, thus the enzymes may be needed to increase fertilization ability (Ciereszko et al. 2000).

To our knowledge, no data are available on the activity of enzymes in *A. persicus* semen. This study was designed to measure the enzymatic activity of AcP, LDH, AS, and *β-N*-AGase and the total protein concentration (TP) in Persian sturgeon semen and to determine the range of enzymatic activity present in the seminal plasma of fresh semen and supernatants obtained after freezing–thawing.

## Methods

### Fish, spermiation induction and sampling

Eight wild-caught Persian sturgeon (143.38 ± 5.47 cm total length and 15.81 ± 0.57 kg mean body weight) were captured (using gillnets, length 18 m, width 5.4 m, mesh size 15 cm) from the southwestern part of the Caspian Sea and transported to the Rajaee Sturgeon Hatchery Center (Sari, Mazandaran, Iran; Lat 36°37′N, Long 53°05′E) between March and April 2011. To induce spermiation, fish were injected with luteinizing hormone-releasing hormone agonists at a dosage of 5 μg/kg body weight (Nazari et al. 2010; Aramli et al. 2014) by placing the syringe in the muscles between the dorsal and lateral scutes. Temperature of the water during the experiment was between 12.5 and 14.0 °C. Spermiation occurred within 12 h after hormonal injection, and followed by subsequent stripping at 17 and 22 h after hormone injection. Semen was collected using a syringe with attached rigid tubing inserted into the urogenital opening. Semen samples (three samples per male) were taken from each of the eight treated males equally during experiment.

### Preparation of seminal plasma and supernatants

Semen was stored in a glass tube on ice (4 °C) during transportation to the laboratory (for up to 3 h). Seminal plasma from fresh semen was separated immediately from spermatozoa by centrifugation (5,000 rpm for 10 min, Labnet Spectrafuge 16 M, Woodbridge, NJ, USA). A part of the semen, which was transferred into 1.5 mL microtubes, was frozen at −79 °C for 1 day to evaluate the extent of leakage of enzymes that occurs after damage to sperm caused by freezing and thawing. Semen was thawed at room temperature, centrifuged and the supernatant was used for enzyme analysis. We did not use any cryoprotectants or extenders in this study.

### Analytical procedures

Acid phosphatase activity was determined using 5 mM p-nitrophenylphosphate in 20 mM citrate buffer, pH 5 (Glogowski et al. 1996). After 30 min of incubation at 37 °C, the reaction was stopped with 0.1 M NaOH, and absorbance at 410 nm was measured (Glogowski et al. 1996). Lactate dehydrogenase activity was measured using the UV method with pyruvate and NADH. The reaction mixture consisted of 1.6 mM sodium pyruvate and 0.2 mM NADH in 80 mM Tris–HCl buffer containing 200 mM NaCl, pH 7.3. Absorbance at 339 nm was measured. The duration of incubation was 5 min at 30 °C (Vassault 1983). Activity of AS was determined using 0.2 ml 20 mM p-nitrocatechol sulfate as a substrate in 0.5 M sodium acetate buffer, pH 4.9. After 30 min of incubation at 37 °C, the reaction was stopped with 2.5 ml 1 M NaOH, and absorbance at 515 nm was measured (Yang and Srivastava 1974). Activity of *β-N*-AGase was measured using 0.5 mM p-nitrophenyl *β-N*-glucosaminide as a substrate in 0.1 M citrate buffer, pH 5.0. After 60 min of incubation at 37 °C, the reaction was stopped with 0.1 M NaOH, and absorbance at 410 nm was measured (Jauhiainen and Vanha-Perttula 1986). Total protein concentration was measured following Lowry et al. (1951), with absorbance measured at 546 nm and 37 °C.

### Statistical analysis

Data were analyzed using one-way analysis of variance followed by Duncan's multiple range test. $P < 0.05$ was considered to be statistically significant. All data are expressed as mean ± standard error.

## Results and discussion

Table 1 shows the TP and enzymatic activities from Persian sturgeon semen. AS, *β-N*-AGase, LDH, and AS activity and protein were detected in both seminal plasma of fresh and frozen–thawed semen. Almost all parameters analyzed in seminal plasma after freezing–thawing were statistically higher than those in the seminal plasma of fresh semen.

Seminal plasma proteins play a key role in prolongation of sperm viability in teleost fish (Lahnsteiner et al. 2004). Thus, a better understanding of the enzyme activities in seminal plasma and spermatozoa is necessary to identify proteins that can be used as indicators of damage to sturgeon semen. Currently, little is known about the proteins present in the seminal plasma of sturgeons. However, specific proteins should exist in the seminal plasma to protect the spermatozoa. Several studies have been conducted to measure the protein concentrations in the seminal plasma of different sturgeon species (Table 2). Similar to most sturgeons, the seminal plasma of Persian sturgeon in contrast to that of higher vertebrates is characterized by much lower protein concentration.

AS and *β-N*-AGase are among the key enzymes localized within the mammalian acrosome that play a pivotal role in penetration of the oocyte (Nikolajczyk and O'Rand 1992; Brandon et al. 1997). In this study, we found that these two enzymes are present in the semen of Persian sturgeon. Their presence was expected due to the presence of the active acrosome in sturgeon semen. Previous results indicated that activities of AS and *β-N*-AGase differed between seminal plasma of fresh and frozen–thawed semen, with greater activity in the latter (Tables 1, 2). In seminal plasma samples of different types of sturgeon, higher concentrations of these enzymes also were present in the supernatant of the frozen–thawed sperm samples (Table 2). These results indicate that these enzymes are localized in both seminal plasma of fresh and frozen–thawed semen (Piros et al. 2002; Sarosiek et al. 2004).

Another enzyme present in the seminal plasma of sturgeon is AcP (Piros et al. 2002). The concentration of this enzyme was very low in the seminal plasma of fresh semen but significantly higher in the supernatant of frozen–thawed semen (Table 2). Sarosiek et al. (2006) reported the existence of at least two forms of AcP with molecular weights of 39 and 19 kDa in the semen of Russian sturgeon. Acid phosphatase role in sturgeon reproduction is unknown. In mammals, AcP has an important role in phosphorylation and dephosphorylation reactions during sperm capacitation, hyperactivation, and acrosome reaction as well as sperm–egg fusion (Urner and Sakkas 2003). Lactate dehydrogenase is an enzyme that helps to produce energy. In fact, formation of lactate allows glycolysis to proceed, in the absence of oxygen, for another cycle of the reaction catalyzed by

**Table 1** Protein concentration and enzymatic activities in seminal plasma of fresh and freeze–thawed semen of Persian sturgeon ($n = 8$ and three samples per male)

| | Fresh | Freeze–thawed |
|---|---|---|
| Protein concentration (g $l^{-1}$)* | 0.28 ± 0.031 | 1.68 ± 0.1 |
| Lactate dehydrogenase (U $l^{-1}$)* | 484.4 ± 27.3 | 6,262.7 ± 263.2 |
| Acid phosphatase (U $l^{-1}$)* | 0.59 ± 0.035 | 9.89 ± 0.81 |
| Arylsulfatase (U $l^{-1}$)* | 169.1 ± 44.4 | 699.6 ± 25.1 |
| *β-N*-acetylglucosaminidase (U $l^{-1}$)* | 36.31 ± 2.25 | 70.7 ± 2.24 |

* Significant different between seminal plasma of fresh and freeze–thawed semen

**Table 2** Comparison of protein content and enzyme activities of seminal plasma from different sturgeon species

| Species | Semen type | TP (g $dl^{-1}$) | AS (U $l^{-1}$) | *β*-N-AGase (U $l^{-1}$) | AcP (U $l^{-1}$) | LDH (U $l^{-1}$) | Authors |
|---|---|---|---|---|---|---|---|
| Sterlet | Fresh | 0.5 | 207.0 | 53.68 | 1.85 | 761.7 | Piros et al. (2002)[a] |
| | Frozen–thawed | 1.33 | 760.2 | 79.09 | 7.89 | 8,803.0 | |
| | Fresh | 0.24 | 54.1 | 23.65 | 0.9 | 71 | Piros et al. (2002)[b] |
| | Frozen–thawed | 1.6 | 937.1 | 44.59 | 5.82 | 9,493.0 | |
| Siberian sturgeon | Fresh | 0.58 | 684.3 | 16.39 | 1 | 278.4 | Piros et al. (2002)[c] |
| | Frozen–thawed | 4.33 | 2,720.5 | 61.59 | 20.6 | 4,420.0 | |
| | Fresh | 0.57 | 638.5 | 20.49 | 1.21 | 400.9 | Piros et al. (2002)[d] |
| | Frozen–thawed | 4.40 | 2,993.9 | 67.28 | 23.81 | 7,217.3 | |
| Stellate | Fresh | 0.3 | | | | | Li et al. (2011) |
| | Frozen–thawed | | | | | | |
| Russian sturgeon | Fresh | 0.6 | | | | | Li et al. (2011) |
| | Frozen–thawed | | | | | | |
| Persian sturgeon | Fresh | 0.28 ± 0.031 | 169.1 ± 44.4 | 36.31 ± 2.25 | 0.59 ± 0.035 | 484.4 ± 27.3 | Present study |
| | Frozen–thawed | 1.68 ± 0.1 | 699.6 ± 25.1 | 70.7 ± 2.24 | 9.89 ± 0.81 | 6,262.7 ± 263.2 | |

*TP* total protein, *AS* arylsulfatase, *β-N-AGase β-N*-acetylglucosaminidase, *AcP* acid phosphatase, *LDH* lactate dehydrogenase

[a] Fish captured from the Danube River

[b] Fish captured from the Dolna Odra Power Plant

[c] 1st Semen collection

[d] 2nd Semen collection

GAPDH (Miki 2007). Like the other enzymes investigated in this paper, LDH activity was higher in the supernatant of frozen–thawed semen than in fresh samples. Piros et al. (2002) also reported a significant increase in LDH in the supernatant of frozen–thawed semen of Siberian sturgeon and sterlet (Table 2). They suggested that the LDH likely originated from spermatozoa and the disruption of the energy supply during cryopreservation, which subsequently affects sperm motility.

According to the data presented in Tables 1 and 2, the activities of all enzymes were much higher after freezing–thawing and significantly higher in the supernatant of frozen–thawed semen. For this reason, they are good potential candidates as markers of cryoinjury, as was previously described for teleost fish (Glogowski et al. 1996; Lahnsteiner et al. 1996; Babiak et al. 1997). Lactate Dehydrogenase and AcP activities may be indicators of damage to the plasmolemma and the midpiece area of the sperm structure, whereas AS and *β-N*-AGase activities may be useful indices of damage to the acrosome in cryopreserved semen. However, further

studies are necessary to explore the roles of these enzymes in the semen structure of Persian sturgeon. In conclusion, the results contribute data on the enzymatic properties of semen of Persian sturgeon. These data are critical for the development of optimal diluents for the long- and short-term storage of sperm in the endangered species.

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**Conflict of interest** The authors declare that they have no conflict of interests.

**Authors' contributions** MRK and MSA participated in the design of the study. MSA and RMN carried out the experiment and performed thestatistical analysis. MSA and MRK participated in the discussion and corrected the manuscript. All authors read andapproved the final manuscript.



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