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Effect of short-term storage on quality parameters, DNA integrity, and oxidative stress in Russian (*Acipenser gueldenstaedtii*) and Siberian (*Acipenser baerii*) sturgeon sperm

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ABSTRACT

The sperm of Russian sturgeon (*A. gueldenstaedtii*) and Siberian sturgeon (*A. baerii*) was used to evaluate the effects of short-term (liquid) storage on functional parameters (spermatozoa motility and velocity), DNA integrity and oxidative stress indices. Spermatozoa showed >50% motility during 6 days of storage with an average velocity of 133.12 ± 15.4 to 87.9 ± 11.23 μm s^{-1} in both species. No motile spermatozoa were recorded after nine days of storage. Analysis of Russian sturgeon sperm showed no significant differences in DNA damage expressed as percent tail DNA and Olive Tail Moment for first three days of storage. In Siberian sturgeon significant differences in DNA damage were detected after two days of storage. The level of oxidative stress indices (TBARS, CP) and antioxidant activity (SOD) increased significantly with storage time in both species. Results of this study can be utilized for successful reproduction management and cryopreservation protocols of these endangered species.

1. Introduction

Sturgeon is among the world's most valuable wildlife resources. These northern hemisphere fishes can be found in large river systems, lakes, coastal waters, and inner seas throughout Eurasia and North America (Birstein and DeSalle, 1998). Most of the world's sturgeon populations have experienced significant decline, mainly due to overfishing, habitat destruction, and pollution (Pikitch et al., 2005). At the same time, sturgeon has become a popular species for aquaculture production. The major hindrance to the culture of sturgeon juveniles is the challenge in simultaneously obtaining viable gametes of both sexes.

To exceed this limitation, various sperm storage methods are frequently used in fish farms. Short-term or liquid storage is the most common technique. Non-diluted sperm storage (Park and Chapman, 2005), sperm storage after dilution with simple ionic medium (Glogowski et al., 2002) or storage after dilution with multi-component media containing a big range of additives such as antibiotics (Brown and Mims, 1995) or antioxidants (Stoss, 1983) are applied as different methodological approaches. In general, sperm is maintained at 4 °C either with aeration or in the presence of CO_2 for several days before fertilization ability decreases (Billard et al., 2004). In aquaculture, refrigerated storage is a simple and inexpensive procedure often needed to deal with logistics of large-scale hatchery operations. Cryopreservation is a powerful tool that allows sperm to be stored indefinitely. This method has been recognized as the most appropriate way for gene banking aimed to conserve specific genetic diversity (Cabrita et al., 2010).

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Any sperm storage procedure must assure the intact preservation of sperm DNA integrity. Several studies have claimed that DNA damage can occur during cold as well as frozen storage, both in mammals and fish (Fraser et al., 2007; Li et al., 2008a), and these DNA alterations may have consequences for the development of offspring (Kopeika et al., 2004). Identification and characterization of DNA damage events could allow storage procedures to be improved by modulating protocols with the objective of diminishing DNA fragmentation.

DNA fragmentation during sperm storage has been ascribed to oxidative stress (Li et al., 2010b). This is a detrimental process affecting spermatozoa structure and function, from plasma membrane composition to the mitochondria and cytoskeleton. It is known that DNA integrity can be impaired in different ways by oxidation. Strand breaks can be generated and nitrogen bases oxidized (Box et al., 2001). Moreover, DNA damage in spermatozoa could be caused by many factors, from sperm aging (Catriona et al., 2011) to the effects of the freezing process (Li et al., 2010c) or UV irradiation (Dietrich et al., 2005). Spermatozoa are sensitive to such stresses, because they possess limited endogenous antioxidant protection while presenting abundant substrates for free radical attack in the form of unsaturated fatty acids and DNA (Koppers et al., 2010). When the production of reactive oxygen species (ROS) by the sperm mitochondria is excessive, the gamete's limited endogenous antioxidant defenses are rapidly overwhelmed and oxidative damage induces lipid peroxidation in the spermatozoa, with a resultant loss of fertilizing potential and vitality (Aitken et al., 1998). Oxidative damage has an important impact on sperm physiology and the study of its nature and effects is of great importance in the field of gamete biology. It will be useful to precisely identify whether oxidative stress occurring during sperm storage affects DNA integrity and whether other mechanisms are involved.

In our study storage of undiluted sperm was used to reveal the possible sources of sperm quality degradation during *in vitro* storage. We evaluated the effect of short-term storage on the physiology and DNA integrity of Russian and Siberian sturgeon sperm with respect to spermatozoa motility and velocity and oxidative stress.

2. Material and methods

2.1. Broodstock handling and collection of gametes

The breeding and culture of Russian sturgeon and Siberian sturgeon were carried out at Fischzucht Rhonforelle GmbH & Co.KG, Gersfeld, Germany. Six males of *A. gueldenstaedtii* (6–8 kg, 7 years old) and six males of *Acipenser baerii* (5–6 kg, 6 years old) were used. Prior to hormone stimulation, fish were kept in tanks with water temperature varying between 14 and 15 °C. Spermiation in both species was stimulated by intramuscular injection of carp pituitary powder dissolved in 0.9% (w/v) NaCl solution at 5 mg/kg of body weight, 48 h before sperm collection. Semen was collected from urogenital papilla by aspiration through a plastic catheter (5–7 mm diameter) connected to a 20 mL syringe. Special care was taken to avoid contamination with mucus, feces, or water. Sperm samples were stored on ice (0–4 °C) until processing.

2.2. Experimental protocol

To investigate the effect of short-term (liquid) storage of sturgeon spermatozoa on DNA integrity, motility characteristics, and oxidative stress indices, the experimental protocol for both Russian sturgeon (*A. gueldenstaedtii*) and Siberian sturgeon (*A. baerii*) was designed to follow standard protocols that are applied for short-term storage of sturgeon spermatozoa under hatchery conditions as follows:

Sperm samples from each species ($n = 6$ per each species) were divided into 250 mL cell containers and kept under aerobic conditions at 4 °C. From each sample, aliquots were removed at 24 (1 day), 48 (2 days), 72 (3 days), 144 (6 days), and 216 (9 days) h after collection, for assessment of functional parameters of sperm, including spermatozoa motility and velocity and DNA integrity, as well as oxidative stress indices and antioxidant activity.

2.3. Sperm motility and velocity recording

Sperm activity was video recorded using dark-field microscopy (Olympus BX 50; stroboscopic lamp Strobex 9630, Chadvick-Helmut, USA) to evaluate motility and velocity. Using a CCD video camera (Sony, SSC-DC50AP), the microscopic field was transferred to a video monitor and recorded with a S-VHS system (Sony, SVO-9500 MDP). The strobe frequency was set to automatic register with video frames (50 Hz) for sperm velocity measurement. Motility and velocity were examined at 20x objective magnification immediately after mixing 1 μL sperm with 49 μL distilled water +0.1% BSA on a glass slide pre-positioned on the microscope stage. The final dilution was 1:10 000. The BSA was added to prevent sperm heads from sticking to the glass slide (Rodina et al., 2007). Within 10 s of mixing, sperm swimming activity was recorded for 2 min. The focal plane was positioned near the glass slide surface.

Successive positions of sperm heads were analyzed from video frames using Olympus MicroImage software (Version 4.0.1. for Windows with a special macro by Olympus C & S). Velocity and percent motility were calculated from sperm head positions on five successive frames with three colors (frame 1 red, frames 2–4 green, and frame 5 blue). Twenty to 40 sperm cells were counted for each frame. Sperm that moved were visible in three colors, while non-moving sperm were white. The percent of motile sperm and sperm velocity was calculated as described (Boryshpolets et al., 2009).

2.4. Oxidative stress and antioxidant indices analyses

Sperm samples were centrifuged at $5000 \times g$ at 4 °C for 10 min. The supernatant was carefully collected and discarded. The spermatozoa pellet was diluted with 50 mM potassium phosphate (KPi) buffer, pH 7.0, containing 0.5 mM EDTA, to obtain a spermatozoa concentration of 5×10^8 cells mL^{-1}, then homogenized in an ice bath using a Sonopuls HD 2070 ultrasonicator (Bandelin Electronic,

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Berlin, Germany). The homogenate was divided into two portions: one in which thiobarbituric acid reactive substances (TBARS) and carbonyl derivatives of proteins (CP) was measured and a second that was centrifuged at 12 000 × *g* for 30 min at 4 °C to obtain the post mitochondrial supernatant for the antioxidant enzyme activity assay.

The TBARS method described by Lushchak et al. (2005) was used to evaluate sperm lipid peroxidation (LPO). Its concentration was calculated by absorption at 535 nm and a molar extinction coefficient of 156 mM cm^{-1}. The content of TBARS was expressed as nanomoles per 10^8 cells. Carbonyl derivatives of proteins were detected by reaction with 2,4-dinitrophenylhydrazine (DNPH) according to the method described by Lenz et al. (1989). The amount of CP was measured spectrophotometrically at 370 nm using a molar extinction coefficient of 22 mM cm^{-1}, expressed as nmol per 10^8 cells.

Total superoxide dismutase (SOD) activity was determined by the method of Marklund and Marklund (1974) and was assessed spectrophotometrically at 420 nm.

2.5. Assessment of DNA damage

The single-cell gel electrophoresis (Comet) assay was performed on the protocol described by Li et al. (2008a). Unless otherwise stated, molecular grade, DNase-free reagents (Sigma Aldrich, USA) were used throughout. Microscope slides (OxiSelectST; Cell Biolabs, INC. USA) were used for Comet assay, and each slide was prepared in the following manner: 50 μL of sperm (6×10^6 cells mL^{-1}) were diluted in 5 mL of PBS (phosphate buffer solution). Diluted samples (200 μL) were mixed with 700 μL of 0.8% NuSieve GTG low melting temperature agarose (OxiSelectST; Cell Biolabs, INC. USA). Finally, 50 μL of this mixture was added to the slide and allowed to solidify for 1 h.

After 1 h the slides were immersed in lysis buffer (2.5 M NaCl, 100 mM EDTA, 10 mM Tris–HCl, 10% dimethylsulfoxide, and 1% Triton X-100, pH 10). The slides were treated with proteinase K (20 mM Tris–HCl, 1 mM CaCl2, and 50% glycerol, pH 7.4) and incubated overnight at 32 °C (Li et al., 2008a). After the proteinase K solution was drained, the slides were immersed in a horizontal gel tank filled with alkaline buffer (300 mM sodium hydroxide, 1 mM EDTA, pH 12.3) for 20 min to allow the DNA to unwind. The buffer level was adjusted to approximately 0.25 cm above the slides, and electrophoresis was carried out for 20 min at 35 V and 170 mA. The slides were drained well, dehydrated by dipping into absolute ethanol for 5 min, and air-dried for storage. For comet visualization 50 μL of Vista Green DNA Staining Solution (OxiSelectST; Cell Biloabs, INC. USA) was loaded onto the slides that were subsequently covered with a coverslip and analyzed with an Olympus BX50 fluorescence microscope at 20× magnifications. A total of 100 cells were scored for each sample, and the captured images were analyzed using CometScore image analysis software (TriTek Corporation, USA). Tail length (measured from the middle of the head to the end of the tail) and tail DNA content (% tail DNA) were measured. Olive Tail Moment (tail length × % tail DNA content) was calculated using the formula below:

$$M_{\text{Tail Olive}} = (|\text{CG}_{\text{Tail}} - \text{CG}_{\text{Head}}|) \times \%\text{DNA}_{\text{Tail}},$$

where $M_{\text{Tail Olive}}$ is the Olive Tail Moment, CG_{Tail} the center of gravity of the tail, CG_{Head} the center of gravity of the head, and % DNA_{Tail} is the percent of migrated DNA in the tail compared to the head.

2.6. Statistical analysis

Normality and the homogeneity of variance of all data were first tested by the Kolmogorov test and the Bartlett test, respectively. Percent motile spermatozoa and spermatozoa velocity were determined for each male ($n = 6$ per each species) at several time points following activation. Measurements were conducted in triplicate. Statistical comparison for percent motile spermatozoa and spermatozoa velocity values was made by analysis of variance (factorial ANOVA) followed by Tukey's HSD test. Data obtained from the Comet assay were log-transformed if necessary. Values include TBARS, CP, SOD level, and percent of DNA damage were expressed as means ± SD. Differences in these values among times of storage for the same species were analyzed by one-way ANOVA, followed by Tukey's HSD test. Differences between species at the same time of storage were analyzed using *t*-test. All analyses were performed at a significance level of 0.05 using STATISTICA 9.0 software for Windows.

3. Results

3.1. Spermatozoa motility and velocity

After sperm activation a majority of sperm samples from males of both species showed 100% motility of spermatozoa within 10 s. During the first three days of sperm storage the percentage of spermatozoa motility displayed the following values: (i) at 10–30 s post-activation, 95–100% of spermatozoa were motile in both analyzed species (Fig. 1A and B) and no significant differences ($P > 0.05$) between fresh and stored samples were observed; (ii) at 40 s post-activation, significant differences ($P < 0.05$) between fresh and stored samples were observed in sperm samples from Russian sturgeon but not in samples from Siberian sturgeon (Fig. 1A and 1B); (iii) during the progress of the motility period, the percentage of actively swimming cells in both analyzed species significantly ($P < 0.05$) decreased with time and it reached 60% at 90 s after activation in both analyzed species (Fig. 1A and B). The period of motility was maintained for 5 min in both species.

A significant decline in percent of motile spermatozoa ($P < 0.05$) was observed after six days of storage for all analyzed samples of both species and percentage of motile spermatozoa demonstrated the following characteristics: (a) at 10 s post-activation 80% of spermatozoa were motile in Russian sturgeon and 78% in Siberian sturgeon, respectively; (b) during the progress of the motility period the percentage of motile spermatozoa decline to 28% at 90 s after activation in Russian sturgeon and to 26% in Siberian

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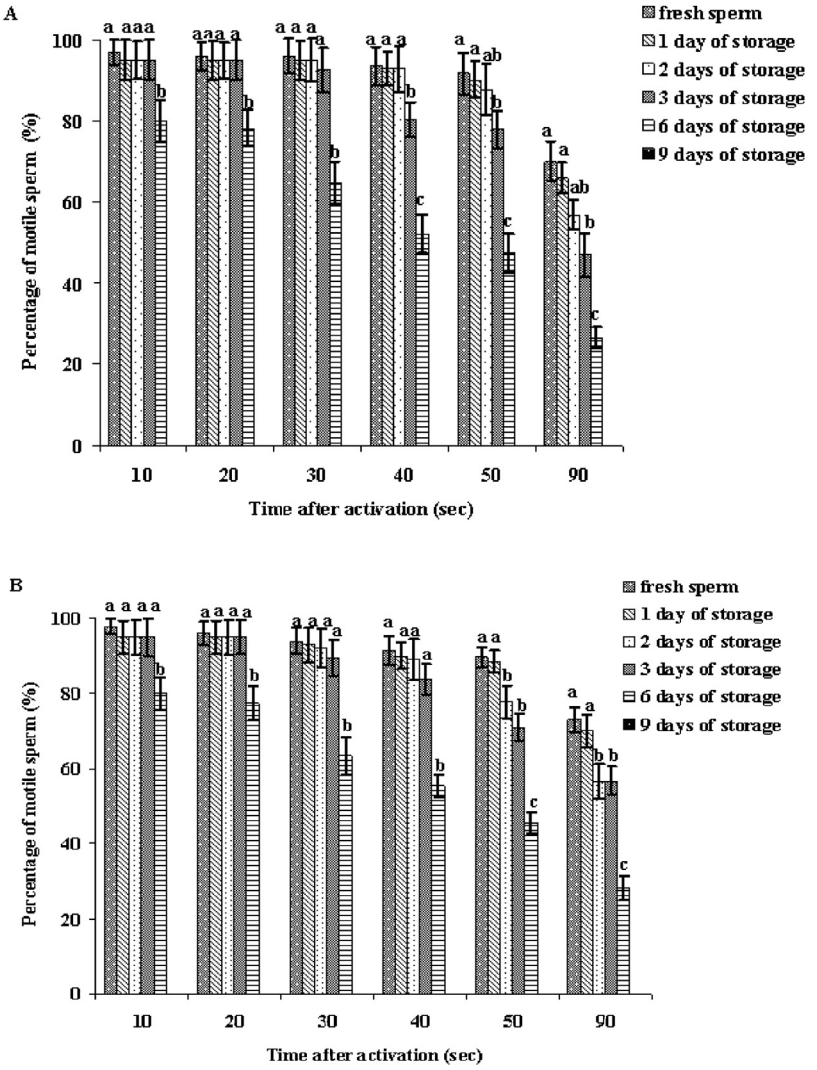

Fig. 1. Effect of short-term storage on *A. gueldenstaedtii* (A) and *Acipenser baerii* (B) spermatozoa motility. Data are presented as means ± SD, $n = 6$ per each species and time of storage. Different letters indicate significant differences among samples (ANOVA, $P < 0.05$).

sturgeon. In addition, a period of motility lasting at 2 min in both analyzed species. After nine days of storage, no motile spermatozoa were observed in either species.

Estimation of spermatozoa curvilinear velocity (VCL) showed a normal distribution and analysis of variance (ANOVA) showed that the significant differences ($P < 0.05$) in spermatozoa velocity between fresh and stored samples were apparent after two days of sperm storage and decline significantly ($P < 0.05$) with time of storage in both species. After the two days of storage, the spermatozoa velocity demonstrated the following characteristics: (a) at 10 s post-activation it reached 173 ± 16 μm/s in *A. gueldenstaedtii* and 165 ± 14 μm/s in *A. baerii* (Fig. 2A and B); (b) after 90 s post activation, the velocity decreased to 105 ± 14 μm/s in Russian sturgeon and 100 ± 13 μm/s in Siberian sturgeon. After six day of storage, the velocity decreased to 133 μm/s at 10 s post activation and to 90 μm/s at 90 s post activation for all analyzed samples of both species.

3.2. Oxidative stress and antioxidant responses

The level of TBARS increased significantly in spermatozoa of Russian sturgeon after six days of sperm storage and after three days of storage in spermatozoa of Siberian sturgeon. No significant differences in level of TBARS between species were observed (Fig. 3A). In addition, the level of CP increased significantly in spermatozoa of Russian sturgeon after two days of storage and in Siberian sturgeon after three days of storage. Moreover, level of CP in spermatozoa was significantly higher in Russian sturgeon comparing to Siberian sturgeon during six days of storage (Fig. 3B). In Russian sturgeon sperm, the measured levels of TBARS and CP varied from 0.33 ± 0.06 to 0.75 ± 0.15 and from 40.1 ± 4.25 to 74 ± 7.3 nmol/10^8 sperm cells, respectively. The antioxidant activity expressed by total SOD activity significantly increased within storage time and no statistical differences in activity of SOD between

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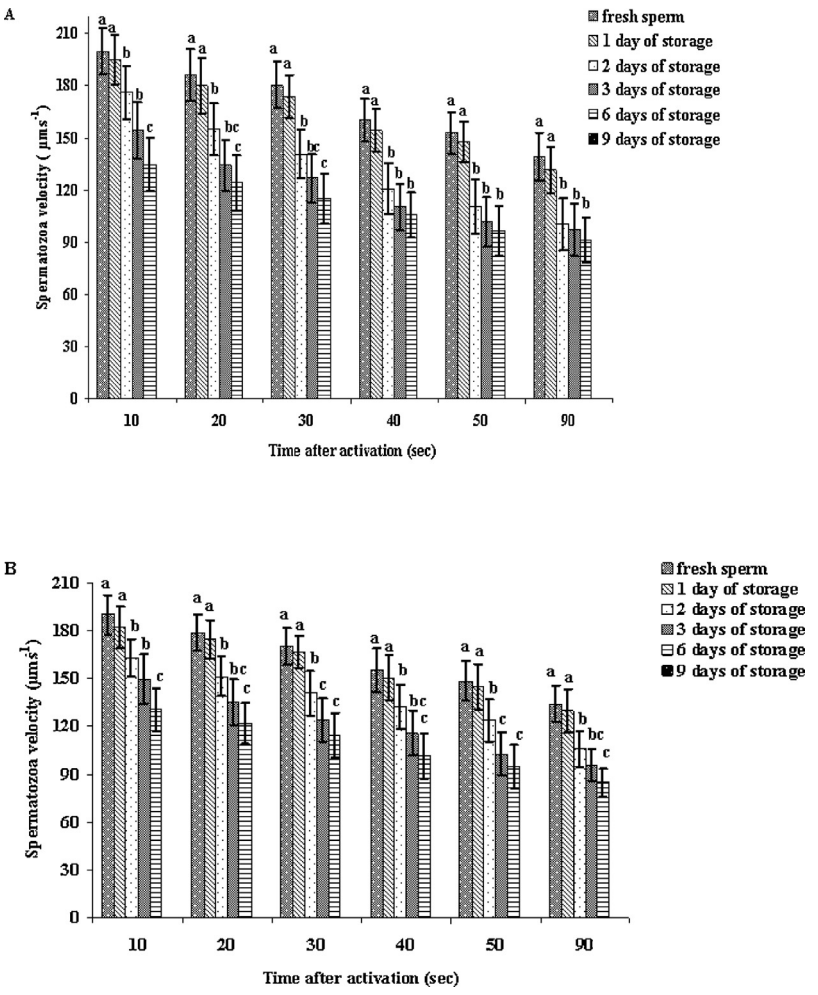

Fig. 2. Effect of short-term storage on *A. gueldenstaedtii* (A) and *Acipenser baerii* (B) spermatozoa velocity. Data are presented as means ± SD, $n = 6$ per each species and time of storage. Different letters indicate significant differences among samples (ANOVA, $P < 0.05$).

Russian and Siberian sturgeon spermatozoa were detected (Fig. 3C).

3.3. Effect of storage on DNA fragmentation

DNA evaluation by Comet assay reported a basal percentage of tail DNA of 5% ± 1.5 in *A. gueldenstaedtii* sperm and 8.5% ± 2.5 in *A. baerii* sperm (Fig. 4A). A significantly higher percentage of tail DNA was found in samples exposed to short-term storage than in fresh ones (22% ± 4.5 in *A. gueldenstaedtii* sperm and 15% ± 5.3 in *A. baerii* sperm). In Russian sturgeon, no significant differences in DNA damage expressed as % tail DNA and Olive Tail Moment during the first 3 days of storage were observed. Subsequently the level of DNA damage in spermatozoa of Russian sturgeon significantly increased. In Siberian sturgeon, the level of DNA damage expressed as % tail DNA and Olive Tail Moment significantly increased after tree days of storage. Significant between-species differences in % tail DNA and Olive Tail Moment were detected after 2 and 3 days, respectively (Fig. 4A and B).

4. Discussion

As a first step toward understanding how short-term storage might alter sperm physiology, DNA integrity, and oxidative stress indices of Russian and Siberian sturgeon sperm, spermatozoa motility and velocity parameters were evaluated.

Our results showed that the spermatozoa of both species were fully capable of being activated immediately after transfer to swimming medium, and 95–100% of spermatozoa became motile. During the first 3 days of storage the percent of spermatozoa motility remained stable in both analyzed species. More specifically, no significant differences between fresh and stored samples during the first 30 s post activation were observed. Moreover, after this period, spermatozoa motility declined. In addition a significant decline in spermatozoa velocity started after two days of storage in both analyzed species. Similar results have been reported in sterlet (*A. ruthenus*) by Dettlaff et al. (1993). Dettlaff et al. (1993) reported that the fertilizing capacity of stored sperm was 5 days during refrigerated storage. In comparison with our study

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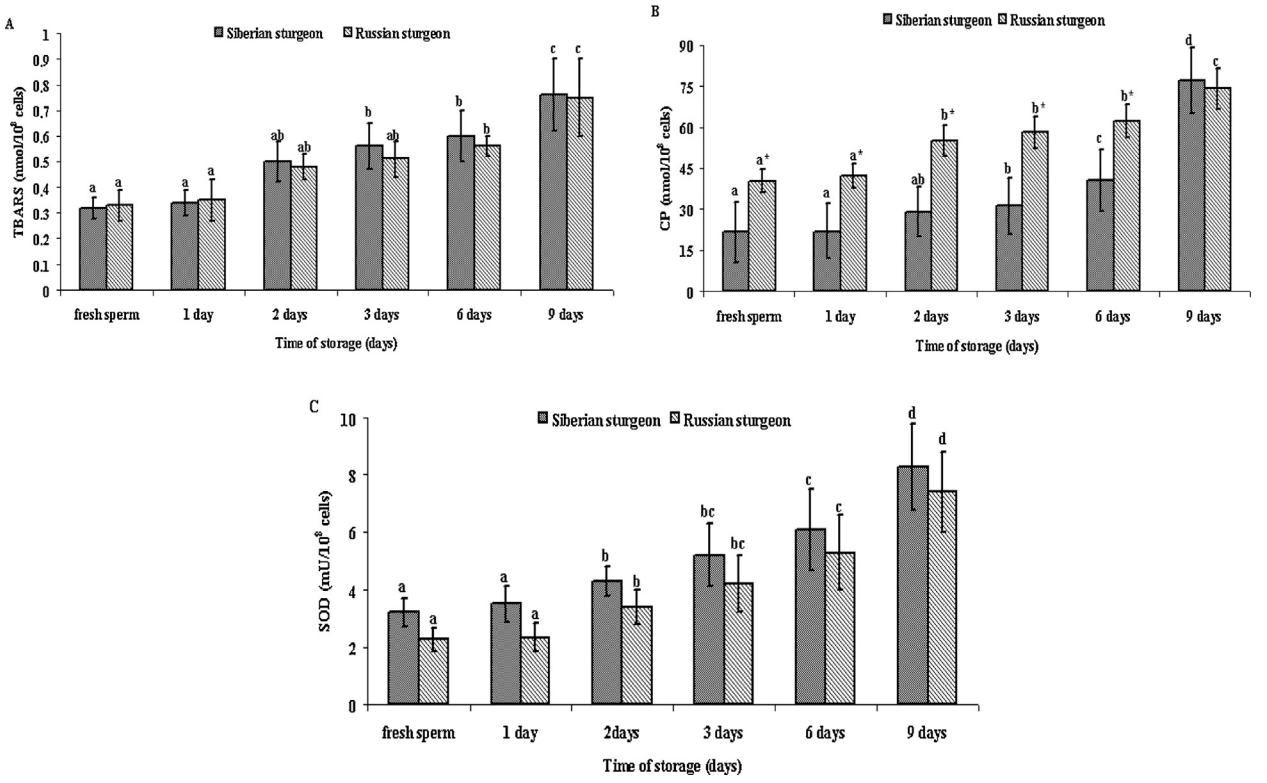

Fig. 3. Effect of short-term storage on oxidative stress indices expressed by TBARS (A); CP (B) and SOD (C) in *A. gueldenstaedtii* and *Acipenser baerii* sperm. Data are presented as means ± SD, $n = 6$ per each species and time of storage. Different letters indicate significant differences among times of storage for the same species (ANOVA, $P < 0.05$); * indicate significant differences between species at the same time of storage (*t*-test, $P < 0.05$).

the sperm of both species were able to become motile during 6 days of storage under aerobic conditions at 4 °C (refrigerated storage). In contrast, DiLauro et al. (1994) reported that in Atlantic sturgeon (*A. oxyrinchus*) 40% of spermatozoa were motile after 17 days storage in plastic bags with daily replenishment of oxygen. Linhart et al. (1995) observed 97% of spermatozoa motility in paddlefish (*Polyodon spathula*) after 16 h storage at 24 °C in a simple saline solution. After nine days of storage, no motility was detected in either of the species in the present study. Oxidative stress has been presumed as the main reason of decline in these functional characteristics (Aitken and Baker, 2006). Disequilibrium between ROS and the spermatozoa antioxidant system can cause metabolic or functional disorders, reducing sperm motility and increased lipid peroxidation (LPO) and carbonylation of proteins (Li et al., 2009).

The lipid composition of the spermatozoa membrane makes it susceptible to oxidative damage, because of their relatively high polyunsaturated fatty acid (PUFA) content (Trenzado et al., 2006). Lipid peroxidation can trigger the loss of membrane integrity, causing increased cell permeability, enzyme inactivation, resistance to osmotic shock, and fertilization potential (Shiva et al., 2011). In addition, protein oxidization could have deleterious effects on sperm function, with plasma membrane structural proteins being affected as well as proteins with enzymatic activity (Domínguez-Rebolledo et al., 2010). Lipid peroxidation is particularly important for aquatic animals, since they normally contain greater amounts of highly unsaturated fatty acids (HUFA) than do other species. Lipid peroxidation has been reported to be a major contributor to the loss of cell function under oxidative stress (Storey, 1996) and has usually been indicated by TBARS in fish (Oakes and Van der Kraak, 2003). Our results showed that the level of TBARS increased significantly after six days of sperm storage in Russian sturgeon and after three days in Siberian sturgeon. In contrast to our results, Li et al. (2010b) found an increasing level of LPO (expressed as TBARS levels) associated with the thawing process after cryopreservation of common carp (*Cyprinus carpio*) spermatozoa, indicating that the ROS generation was primarily related to the freeze/thaw process rather than the effect of equilibration. The CP is a result of protein oxidation. The formation of CP is nonreversible, causing conformational changes, decreased catalytic activity in enzymes, and ultimately resulting, owing to increased susceptibility to protease action, in breakdown of proteins by proteases (Zhang et al., 2008). In the present study we observed that the level of CP increased significantly in spermatozoa of Russian sturgeon after two days of storage and in Siberian sturgeon after three days of storage. Subsequently, the level of CP significantly increased with storage time. Based on our results, we hypothesize that in Russian sturgeon and Siberian sturgeon the cause of the loss of spermatozoa motility and velocity during short-term storage was oxidative stress that significantly interrupted

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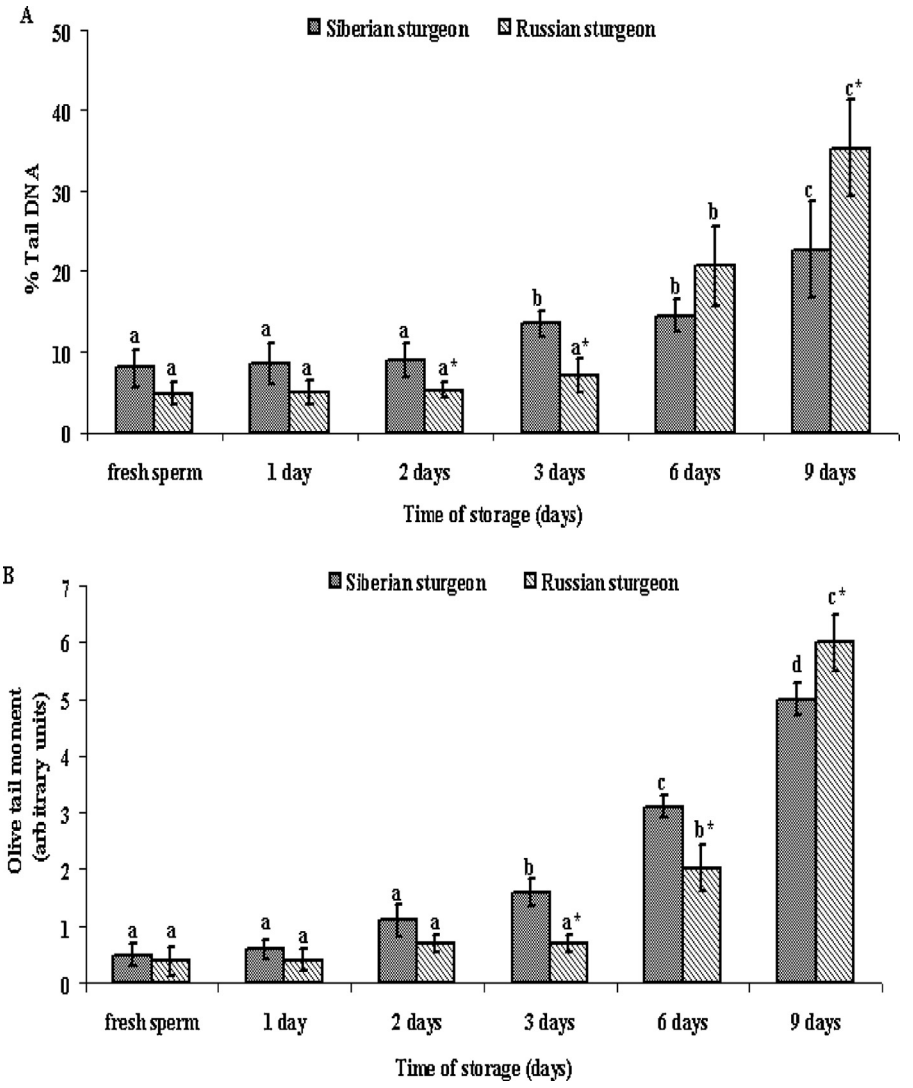

Fig. 4. Percent of tail DNA (A) and Olive Tail Moment (B) in *A. gueldenstaedtii* and *Acipenser baerii* sperm, during short-term storage ($n = 6$ per each species). Columns represent mean values from 600 cells, 100 per male. Data are presented as means ± SD. Different letters indicate significant differences among times of storage for the same species (ANOVA, $P < 0.05$); * indicate significant differences between species at the same time of storage (t-test, $P < 0.05$).

cellular metabolism (*e.g.* oxidative phosphorylation) of spermatozoa, leading to subsequent decline of motility parameters. Additionally, in our study we observed significant differences in the level of CP between species, but future investigation is required for the elucidation of the reasons.

Fish sperm possess an antioxidant system consist of enzymatic (glutathione peroxidase and reductase, catalase, superoxide dismutase) and non-enzymatic (α-tocopherol, ascorbic acid, β-carotene, selenium, zinc) components that are capable to counteract the damaging effects of ROS and protect the cell structure (Li et al., 2009). However, a limited amount of information is available about the precise mechanism of action of antioxidant systems in fish sperm (Lahnsteiner et al., 2010). In the present study the antioxidant activity expressed by total SOD activity was significantly increased after two days storage in sperm of both species. However, Li et al. (2010b) showed that SOD activity in carp spermatozoa was neither activated nor inhibited by the freeze/thaw process, indicating that the antioxidant defense system in spermatozoa is species specific. Based on our results we argue that the level of antioxidant enzymatic activity is insufficient to prevent cellular damage and DNA fragmentation caused by oxidative stress resulting from short storage during more than three days in Russian sturgeon and two days in Siberian sturgeon, respectively. Several studies, have demonstrated that it is possible to reduce damaging effects of ROS by the addition of various antioxidant compounds to the freezing media prior to cryopreservation of fish sperm (Lahnsteiner et al., 2011). However, the effect of each antioxidant is species-specific, improving different parameters of sperm quality depending on the type of antioxidant and concentration used (Cabrita et al., 2011). Therefore we argue

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that as a future step in investigation of various effects of short term or cold storage on sturgeon spermatozoa should be focused on the application of antioxidants that will be capable of preventing deleterious effects of storage on qualitative sperm parameters during the short term storage or cryopreservation.

In the present study, the Comet assay method was used to evaluate the effect of short-term sperm storage on DNA integrity. Our results showed that during short-term storage no significant differences in DNA damage expressed as percent tail DNA and Olive Moment during the first three days of storage of Russian sturgeon sperm were observed. However results recorded after six days of storage showed a significant increase in level of DNA damage. In sperm of Siberian sturgeon the significant increases in DNA damage were observed after three days of storage. Pérez-Cerezales et al. (2009) reported that classical cold storage procedures (~2 h at 4 °C with aeration) significantly increased DNA damage in rainbow trout (*Oncorhynchus mykiss*) sperm. Labbe et al. (2001), using the Comet assay, demonstrated that the DNA stability in rainbow trout sperm was slightly affected by cryopreservation. Cabrita et al. (2005), using the same method, found a significant increase in fragmented DNA in cryopreserved rainbow trout sperm, while in gilthead sea bream (*Sparus aurata*) varying effects on DNA integrity were related to differing dilutions following cryopreservation. The differences observed in the abovementioned studies could be also related to the chromatin structure of the analyzed species or the obtaining of the sperm at different times during the reproductive season. Differences between species detected in our study in the level of DNA damage during short-term storage could not be explained directly from our results because a little information in this field is available. Additional experiments are required to investigate possible existence of species-specific cellular mechanisms responsible for stability of spermatozoa chromatin during *in vitro* storage.

In conclusion, the results of the present study provide new data on Russian and Siberian sturgeon sperm quality with respect to short-term storage and indicate that the decline in sperm quality can provide novel information about integrity of the DNA molecule is likely caused by oxidative stress and accumulation of LPO and CP in sperm cells resulting from cold storage of sperm. The data presented here suggest that application of antioxidants during liquid storage or cryopreservation of fish spermatozoa could prevent cellular injuries caused by oxidative stress.

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