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RESEARCH PAPER

Standardized Sperm Cryofreezing Technique for Caspian Sea Sturgeons (*Huso huso*, *Acipenser persicus*, *Acipenser stellatus*, *Acipenser nudiventris*)

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Abstract

Sturgeons, considered "living fossils," face extinction due to various factors, according to the International Union for Conservation of Nature's Red List. The present study describes a uniform sperm cryopreservation method for the endangered Caspian Sea sturgeon species, which are beluga (*Huso huso*), Persian (*Acipenser persicus*), stellate (*Acipenser stellatus*), and ship (*Acipenser nudiventris*) sturgeons for both artificial reproduction and *ex-situ* conservation attempts. For this aim, sperm samples were diluted 1:1 in extender composed of 118 mM Tris buffer (pH=8) and 23.4 mM sucrose-based extender containing 15% DMSO and equilibrated for one hour at 4°C. Then, sperm samples were placed into 0.50-ml straws and multi-stage cooling was applied to freeze with the help of a programmable freezer with an accuracy of ±0.1°C/min, and finally stored in liquid nitrogen (-196°C) tank. Thawing was performed at 40°C water baths for 25 s. The results indicated that storage for two years resulted in a 24.7% fertilization rate for the stellate sturgeon, while the Persian sturgeon had a remarkable rate of 58.2% after five years storage.

Introduction

The Caspian Sea, known as the largest brackish water lake globally, houses six species of sturgeons. The Caspian Sea has long been recognized as the sturgeon sea due to its contribution of over 90 percent of the global caviar supply. Even though sturgeon numbers have dropped significantly recently, the Caspian Sea remains a vital location for sturgeon fishing and aquaculture.

Sturgeon populations and their diversity have suffered declines as a result of environmental pressures like water pollution, dam building, and the development of nearby watersheds for irrigation. Sturgeons, currently known as 'living fossils', are at risk of extinction based on the Red List created by the International Union for Conservation of Nature (Bozkurt & Chebanov, 2024). It is apparent that the standard approaches of reducing pollution and conserving habitats have not been effective in preserving and replenishing these populations. Thus, there is an urgent need to develop more stringently measures for the conservation of sturgeon stocks (Pourkazemi, 2006). In light of the potential extinction of sturgeon in the Caspian Sea, it is crucial to preserve their sperm through *ex-situ* methods to ensure their long-term conservation. Thus, biotechnological tools such as cryopreservation should promptly be integrated into ex-situ conservation initiatives such as cryobanking.

Burtsev and Serebryakova made the first attempt to preserve the sperm of three sturgeon species: *Huso huso* (Beluga), *Huso dauricus* (Kaluga), and *Acipenser ruthenus* (Sterlet sturgeon) (Mims et al., 2011). Since then, many studies carried out on cryopreservation of sperm in different sturgeon species including *Acipenser fulvescens* (Lake sturgeon), *Acipenser baeri* (Siberian sturgeon), *Acipenser sturio* (Atlantic sturgeon), *Acipenser sinensis* (Chinese sturgeon), *Acipenser persicus* (Persian sturgeon), *Acipenser stellatus* (Stellate sturgeon), *Acipenser gueldenstaedtii* (Russian sturgeon), *Acipenser transmontanus* (White sturgeon), and *Polyodon spathula* (Paddlefish) (Yamaner et al., 2015). On the other hand, a uniform sperm cryopreservation study including all Caspian Sea sturgeon species has never been studied to date, which is the main motivation of this study.

Preserving sperm through cryopreservation is a crucial step in advancing biotechnology for assisted reproduction in fish (Bozkurt, 2019). In this regard, cryopreserved sperm enables the creation of gene banks and facilitates the combination of genetic traits for endangered species. Additionally, the use of cryopreserved sperm in breeding initiatives can help in the creation of improved genetic stocks and the develop of genetic reserves for aquaculture.

Gamete cryopreservation requires the collection of gametes, evaluation of initial sperm quality, semen dilution in an cryosolution, equilibration of diluted sperm, freezing of sperm, thawing of frozen sperm, and evaluation of sperm quality post-thawing (Asturiano et al., 2017; Martinez-Paramo et al., 2017). Several methods have been established for freezing sturgeon sperm. These investigations have emphasized the importance of selecting the optimal cryoprotectant, extender, and their combinations; dilution rate, freezing and thawing methods etc. (Dzyuba et al., 1999; Billard et al., 2004, Kopeika et al., 2008). The cryopreservation methods currently used are not sufficiently efficient for regular use in sturgeon breeding and genetic material preservation (Beirao et al., 2019; Contreras et al., 2019).

In this regard, one challenge in semen cryopreservation is the need to significantly adjust methods to different species, even in the same genus. Although there have been advancements in milt cryopreservation in recent decades, the outcomes tend to be inconsistent, requiring the customization techniques for individual species. In this context, because sturgeons exhibit a high degree of similarity in sperm biology, cryopreservation techniques can be transferred between different species with minimal protocol adaptations (Horvath et al., 2011).

In the context of this study, we report how fertilization was affected by the use of sturgeon sperm frozen by a simple protocol using computer-assisted freezing. We also tested the effectiveness of DMSO as cryoprotectant together with sucrose-Tris based solution as an extender. We choosed sucrose solution because sperm from aquatic organisms with acrosomes could be effectively preserved using this solution according to some authors (Glogowski et al., 2002; Mims et al. 2000).

From this point of view, this research is focused on testing a consistent sperm cryopreservation method to support gene banking initiatives for the endangered Caspian Sea sturgeon species such as beluga (*Huso huso*), Persian sturgeon (*Acipenser persicus*), stellate (*Acipenser stellatus*), and ship (*Acipenser nudiventris*). The efficacy of cryopreservation protocols was determined by evaluating the fertilization ratio of thawed sperm.

Material and Methods

Broodstock

The broodstock were caught using gillnets from the Sefid Rud River in the north of Iran during the breeding periods of April-June 2015 and 2016. Captured 34 male sturgeon broodstock, including 25 males of *A. persicus*, four males of *A. stellatus*, three males of *H. huso*, and two males of *A. nudiventris* were transported to the Shahid Beheshti Sturgeon Hatchery located in Rasht, Iran. However, 18 male brood sturgeons were included to the study due to their high quality sperm.

The sturgeon broodstock were kept in separated tanks for 30 days for maturation before artificial propagation experiments. Depending on the species, the water temperature conditions for keeping the broodstock were in the range of 10-14°C and dissolved oxygen was more than 7.5 ppm and water pH was between 5.5-7.5 during this period. The guidelines for the care and use of animals for scientific purposes were followed during fish manipulations (National Health and Medical Research Council, Australia).

Gamete Collection

For the aim of hormone injection and gamete collection, the male and female breeders were anesthetized using clove powder solution at the rate of 150-200 mg/L. Sexually mature broodfish were intramuscularly induced to spawn by injections of GnRH hormone (Samen company, Mashhad, Iran). For this aim, this hormone was injected to the female broodfish in the amount of 20 µg kg^{-1} body weight in two stages, in the first stage 20%, and after 6 hours the remaining hormone was injected inside of the back muscle. For spermiation of the male broodfish, the GnRH hormone injection was done at the same time as the second injection of the female broodfish at the rate of 10 µg kg^{-1} body weight 24 h before stripping (Eenennaam et al., 2008).

Sperm was collected from the urogenital papilla by aspiration using dry syringe (50 ml) with a silicon tube attached to it, after cleaning and drying the genital region to avoid contamination of mucus, feces, or water. The collected sperm samples were kept in 15 ml falcon

tubes and instantly stored in a refrigerator at a temperature of 5°C. Then, the fresh sperm in falcon tubes were transferred to the laboratory immediately to evaluate its quality in terms of motility, density, pH and colour.

Eggs were collected from two females of each species 24 h after hormone injection by inverting and applying slight pressure to the abdominal region. The eggs flowed through the urogenital opening into a plastic bowl. Then, collected eggs were stored in aerobic conditions at 17°C following evaluation in terms of homogeneous shape, colour and size are used for fertilization within 30 min.

Evaluation of Sperm Quality

Fresh and post-thawed spermatozoa (1 µl) were mixed with hatchery water (10 µl) for activation of sperm cells to evaluate spermatozoa motility. Sperm motility was determined by observing the percentage of cells with progressive movement, and the time until this movement ceased was recorded. These parameters were estimated in triplicate visually, using light microscope (Olympus BX50, Tokyo, Japan) under 400x magnification at room temperature condition (20±2°C) (Sarosiek et al., 2004).

The density of sperm cells were determined according to the hemocytometric method. For this aim, sperm were diluted at a ratio of 1:1000 with Hayem solution (35.2 mM Na_2SO_4, 17.1 mM NaCl, 1.8 mM $HgCl_2$, 200 mL bicine), and density was determined using a 100 µm deep Thoma hemocytometer (TH-100; Hecht-Assistant, Sondheim, Germany) at 400x magnification with an Olympus BX50 phase contrast microscope and expressed as sperm $x10^9$ mL^{-1} (three replicates) (Bozkurt & Yavaş, 2024). Sperm density was calculated as follows: Sperm density (per ml) = 1000 x number of counted sperm/[area (mm^2) x chamber depth (mm) x dilution ratio] (Aramli & Nazari, 2014). Spermatocrit was evaluated after centrifugation at 5000 rpm for 5 minutes (Williot et al., 2002). Standard pH electrodes were used to measure sperm pH within 30 min. of sperm collection. All the sperm quality analyses were replicated three times.

Quantity and quality of sperm of 34 male breeders of sturgeon, including 25 males of *A. persicus*, four males of *A. stellatus*, three males of *H. huso*, and two males of *A. nudiventris* were evaluated. However, sperm samples from 18 male brood sturgeon with suitable quality especially having more than 70% motility were selected for the cryopreservation experiments (Table 4). The selected sperm samples were kept at 5°C for the cryopreservation experiments (Park & Chapman, 2005).

Cryopreservation Procedure

Separate samples of sperm were taken from each male and diluted with a 1:1 ratio in cryosolution, which previously cooled at 4°C. The cryosolution was composed of 118 mM Tris buffer (pH=8), 23.4 mM sucrose, and 15% DMSO (Cherepanov & Kopieka, 1999; Dzyuba et al., 1999; Kopieka et al., 2000) and was kept at 4°C for one hour for equilibration. Following, the transfer of the samples into freezing straws with a volume of 0.5 ml was done by an automatic filling machine (IMV France). Then multi-stage cooling (Drokin & Kopieka, 1999) after equilibration the diluted samples, with the help of a programmable freezer with the accuracy of ±0.1°C/min, were implemented for each species separately as described in Tables No. 1, 2, and 3

Table 1. Intensity of cooling in sperm of beluga (Billard et al., 2004)

Cooling stages	Initial temp. (°C)	Final temp. (°C)	Intensity (°C/min)
Stage I	5	-10	2
Stage II	-10	-70	20
Stage III	-70	-90	30
Stage IV	-90	-196	Immediately

Table 2. Intensity of cooling in sperm of ship and stellate sturgeon (Billard et al., 2004)

Cooling stages	Initial temp. (°C)	Final temp. (°C)	Intensity (°C/min)
Stage I	5	-9	3.5
Stage II	-9	-70	25
Stage III	-70	-90	30
Stage IV	-90	-196	Immediately

Table 3. Intensity of cooling in sperm of Persian sturgeon (Noveiri et al., 2006)

Cooling stages	Initial temp. (°C)	Final temp. (°C)	Intensity (°C/min)
Stage I	5	-15	3
Stage II	-15	-70	20
Stage III	-70	-90	25
Stage IV	-90	-196	Immediately

(Glogowski et al., 2002; Linhart et al., 2006; Kopieka, & Kopieka, 2008). The temperature regulation characteristics of the multiple stages of chilling were the same for each species in all experiments. In the last stage, the frozen sperm samples were collected and stored in a liquid nitrogen tank (-196°C) for long-term storage. The spermatozoa motility during the storage of cryopreserved semen samples, at different time intervals after cryopreservation was monitored (Figure 1).

Thawing of Frozen Sperm and Fertilization

The straws were thawed in a water bath at 40°C for 25 s (Tsvetkova et al., 1996; Lahnsteiner et al., 2004) for further evaluation and fertilization. The same sperm-to-egg ratio was used for both fresh and cryopreserved sperm for each species separately during fertilization. The effectiveness of fresh and frozen-thawed sperm in fertilization was assessed by conducting three replicates using eggs obtained from one female spawners for Persian and stellate sturgeons during the breeding season. For fertilization, the ovarian fluid was removed and eggs were placed in a Petri dish. Subsequently, 1 ml of thawed sperm was used for every 100 g of Persian sturgeon eggs, and 1 ml of thawed sperm was used for every 100 g of stellate sturgeon eggs in the fertilization experiments. This procedure was also conducted using fresh sperm with all batches of eggs for each species separately. Then, the eggs were quickly combined with 8 mL of hatcher water used for activating solution. Five minutes later, approximately 50 mL of hatchery water was added again, and the eggs were rinsed multiple times, continuously stirred for ten minutes, and then washed with a tannic acid solution (200 mg L^{-1}) to get rid of stickiness. The eggs were placed into individual plastic baskets with flowing hatchery water and then incubated. Fertilization success was determined at 15-16°C when the eggs reached to the four-cell stage (Dettlaff et al., 1993), which occurred about 24 h following fertilization.

Statistical Analyses

Overall differences between means were considered significant when $p<0.05$. The relationship between post-thaw motility (%) and storage period (day) was carried out using the MS Excel statistical data analysis. Other results were statistically processed using SPSS program.

Results

For fresh sperm, the highest sperm motility ranges (70-90%), sperm density ($2.75\pm1.68x10^9$ mL^{-1}), and spermatocrit levels (8.16±4.3) were measured in *Acipenser persius* whereas, the lowest motility ranges (50-54%) in *Acipenser nudiventris*, sperm density ($0.95\pm0.32x10^9$ mL^{-1}), and spermatocrit levels (1.3±0.4) were measured in *Acipenser stellatus*. According to the results, the lowest post-thaw motility ranges (<30%) were determined in *Acipenser percius* sperm. Also, the highest mean sperm pH was measured in *Acipenser nudiventris* as (9.11%), whereas, its the lowest mean value in *Acipenser percius* as (8.42±0.54) (Table 4).

The post-thaw motility values decreased with storage period. The changing trend of post-thaw motilities were described in Figure 1. The findings from fertilization tests with fresh and cryopreserved sperm are outlined in Table 5. Among the cryopreserved samples, the highest mean post-thaw fertilization (58.2%) were observed in Persian sturgeon. The fertilization rates using thawed-frozen sperm in one beluga and one ship sturgeon were 7% and 8%, respectively.

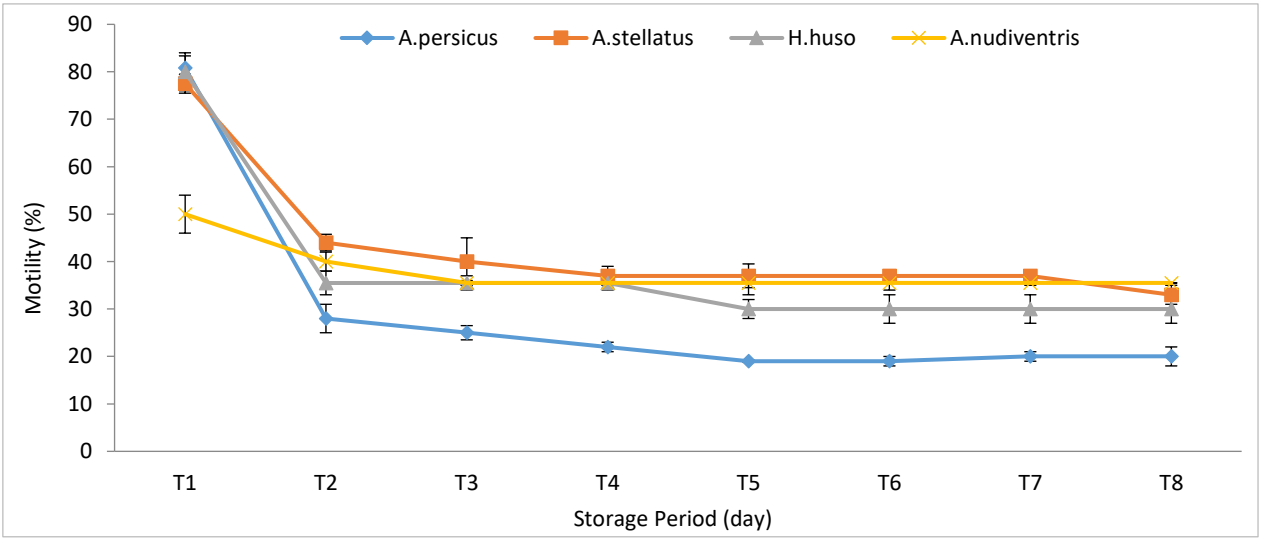

Figure 1. The changing trend of the motilities of Caspian Sea sturgeon broodstock sperm [T1: initial motility, T2: post-thaw motility (immediately), T3: PT motility after 60 days, T4: PT motility after 90 days, T5: PT motility after 120 days, T6: PT motility after 150 days, T7: PT motility after 180 days, T8: PT motility after 210 days].

Table 4. Biometric characteristics and quality parameters of sperm by Caspian Sea sturgeon species.

Caspian Sea Sturgeons	Weight (kg)	Total length (cm)	Fresh Sperm Motility Ranges (%)	Post-thaw Sperm Motility Ranges (%) (210 days storage)	Sperm Density (10^9/ml)	Spermatocrit (%)	Sperm pH	Total Cryopreserved Sperm Amounts (ml)
Acipenser percius (n=12)	17.175±1.4	150.5±7.5	70-90	<30	2.75±1.68	8.16±4.3	8.42±0.54	1010
Acipenser stellatus (n=4)	10.74±2.78	112.75±7.5	70-80	32-45	0.95±0.32	1.3±0.4	8.81±0.88	90
Huso huso (n=1)	83	217	77-80	30-35	2.131	2	8.57	80
Acipenser nudiventris (n=1)	Not measured	Not measured	50-54	35-40	1.116	2	9.11	110

Table 5. The fertilization results using fresh and cryopreserved sperm from Caspian Sea sturgeon broodstock at the end of 210 days cryostorage.

Caspian sturgeon species	Fresh Sperm Fertilization Percentage Ranges (%) (24 hours later following fertilization)	Post-thaw Fertilization Percentages (%) (24 hours later following fertilization)
Acipenser percius	65-70	58.2
Acipenser stellatus	67-70	24.7
Huso huso	70-78	7
Acipenser nudiventris	50-60	8

Discussion

Sperm cryopreservation is a valuable tool for the management and improvement of selective breeding programs and genetic conservation of endangered aquatic species. The establishment of sperm cryobanks for Caspian Sea sturgeon species, which are critically endangered, can help in conserving of natural stocks and in raising of this valuable species. Considering this perspective, this study describes the first attempt to freeze sperm in Caspian Sea sturgeon species using a uniform method for establishing cryobanks.

In spite of there are many studies on cryopreservation of sperm in different sturgeon species such as *Acipenser baerii* (Judycka et al., 2015), *Acipenser ruthenus* (Jahnichen et al., 1999), *Acipenser fulvesscens* (Toth et al., 1999), *Acipenser gueldenstaedtii* (Shaliutina et al., 2013) a uniform sperm cryopreservation study including all Caspian Sea sturgeon species has never been studied. Additionally, limited amounts of data are available regarding this issue and the methods have only been adapted to sturgeons individually.

The variability in extenders, cryoprotectants, and freezing methods complicates the assessment of the effectiveness of different cryopreservation techniques (Bozkurt & Yavaş, 2024). It is possible to indicate that using a basic extender blend, reducing dilution levels, and minimizing holding time makes freezing processes less complex. In this regard, firstly, an effective cryopreservation technique requires a proper extender with an optimal level of cryoprotectant to decrease cell damage linked to dehydration, cellular injuries, and ice crystal formation (Bozkurt & Yavaş, 2024).

In line with cryobiology principles, sugars shield cells from osmotic shock when external water decreases due to ice formation, and they may also aid in preserving the structural and functional integrity of membranes in cold temperatures. In this regard, carbohydrate-based solutions are commonly utilized in research for immobilization of sturgeon semen for cryopreservation (Judycka et al., 2015; Horvath et al., 2011). In fish sperm cryopreservation, saccharides like sucrose are frequently utilized to extend the lifespan of the sperm (Stoss and Holtz, 1983; Ciereszko & Dabrowski, 1996). The carbohydrates are successful due to their ability to protect against freezing.

The results of this study support earlier research indicating that a straightforward sucrose-containing medium is beneficial for freezing sturgeon sperm. In this context, despite glucose usage in sperm cryopreservation studies as in some sturgeon species (Judyca et al., 2024; Aramli et al., 2015), the present study showed that sucrose-Tris based cryosolution provided effective post-thaw motility and fertilization results in combination with the 15% of DMSO for Persian and stellate sturgeon. Similar to our study, Tsvetkova et al., (1996) reported successful post-thaw motility and fertilization results using cryopreserved sperm from sterlet (*Acipenser ruthenus*) and Siberian sturgeon (*Acipenser baeri*) when sperm was diluted in a 1:1 ratio with a cryosolution including 15% DMSO, 23.4 mM sucrose, 118 mM Tris-HCl, 20% egg yolk and thawed for 25 s at 40ºC resulting 23±9% motility and 53±8% fertility in Siberian sturgeon and 15 ± 11% motility and 23±11% fertility in sterlet.

Cryoprotectants exhibit varying protective effects in different fish species. The effectiveness of CPAs in sperm cryopreservation may differ depending on the fish species. The differences in sperm size, shape, and seminal plasma composition, such as ionic and organic components and osmolality, between species might be the reason. For example, while 12.5% or 17.50% egg yolk was used as CPA successfully in sperm cryopreservation in sterlet (Jahnichen et al., 1999), but it was found too toxic for use in Persian sturgeon (Shaluei et al., 2017). DMSO is a commonly used cryoprotectant to lower the freezing point of the extracellular medium, reduce the harm caused by ice crystals, and control the rate of

cellular dehydration in freshwater fishes. It is typically applied at concentrations ranging from 5 to 25%.

Pushkar et al. (1979) have demonstrated that DMSO was the best cryoprotectant for sturgeon sperm when used in a diluent containing Tris-buffer and egg yolk, with 50 to 60% of the post-thawed sperm motility in stellate sturgeon and 64% fertility in Russian sturgeon (*Acipenser guldenstadti)*. Mims et al. (2000) demonstrated that employing DMSO as a cryoprotectant along with a slow, three-stage freezing regimen produced the most favorable outcomes for cryopreserving paddlefish (*Polyodon spathula*) sperm. However, methanol was found to be a viable cryoprotectant for sterlet sperm by Horvath & Urbanyi (2000) in comparison to DMSO and DMA at different concentrations. According to these authors, the most favorable post-thaw motility results (46±23%) and fertility (22±16%) were obtained using the sucrose extender and 10% methanol, whereas DMSO only yielded 2±4% fertility and DMA none.

Taking into account the overall results obtained from these experiments, our findings are in line with earlier studies that suggest DMSO as an efficient CPA for sturgeon (Tsvetkova et al. 1996; Glogowski et al. 2002; Boryshpolets et al. 2011) and paddlefish (Horvath et al. 2006; Linhart et al., 2006) sperm. According to literature, DMSO is known as the most efficient CPA, potentially because of its small molecular size, rapid penetration into sperm cells, and its bonding with the sperm membrane's phospholipids (Suquet et al., 2000).

It is important to note that sturgeon sperm is extremely challenging to preserve through cryopreservation due to its unique properties, such as a short duration of motility, low production of adenosine triphosphate (ATP), and high sensitivity to osmotic stress. These factors make the sperm more susceptible to damage during the cryopreservation process (Martinez-Paramo et al., 2009). Thus, the implemented freezing protocols have great importance among the factors affecting sperm cryopreservation success in sturgeons.

Regarding this issue, Cherepanov & Kopeika (1999) indicated that the best protocol was obtained when 10-15% DMSO used with 100-150 mM Tris-HCl extender including 10-15% chicken egg yolk (pH 8.1) when sturgeon sperm of various species were successfully fertilized using a three-step process to freeze the sperm in polypropylene ampoules ranging from 0.5 to 1.5 mL: *Huso huso, Acipenser baeri, Acipenser gueldenstaedtii gitldenstadtii, Acipenser medirostris, Acipenser nudiventris, Acipenser ruthenus, Acipenser stellatus, and hybrid - Huso huso x Acipenser ruthenus.*

Additionally, Drokin & Kopeika (1999) reported sperm cryopreservation results of Siberian sturgeon (*Acipenser baerii*), Sakhalin sturgeon (*Acipenser mikadoi*), and stellate applying the same protocol, which diluting sperm 1:1 in a cryosolution containing 20 mM Tris-HCl, 15% DMSO, 18% egg yolk (pH 8) pouring into plastic ampoules and freezing in a three-step program. In this freezing protocol, ampoules were thawed at 40°C for 30 s. resulting in post-thaw motility results such as 30-40% for Sakhalin sturgeon, 15-20% for Siberian sturgeon and stellate sperm had 30-40%.

However, according to applied freezing protocol in this study, sperm samples were diluted 1:1 in extender composed of 118 mM Tris-buffer (pH=8) and 23.4 mM sucrose-based extender containing 15% DMSO and equilibrated for one hour at 4°C. Then, sperm samples were placed into 0.50-ml straws and multi-stage cooling was applied to freeze with the help of a programmable freezer with an accuracy of ±0.1°C/min, and finally stored in liquid nitrogen (-196°C) tank. Following thawing of frozen sperm samples at 40°C water baths for 25 s., the results indicated that storage for 2 years resulted in a 24.7% fertilization rate for the stellate sturgeon, while the Persian sturgeon had a remarkable rate of 58.2% after 5 years storage. To the best of our knowledge, this study represents the initial trial of employing a controlled-rate freezer for preserving sperm from Caspian Sea sturgeon species. In earlier experiments, sturgeon sperm was cryopreserved using a polystyrene box with straws placed on different height frames floating on liquid nitrogen. (Abed-Elmdoust et al., 2019; Golshahi et al., 2018; Linhart et al., 2006; Glogowski et al., 2002). However, the implemented technique in the present study provides us with the opportunity to adjust the freezing rate in cryopreservation. This allows for the cryopreservation process to be performed under any circumstances and can be implemented for extensive sturgeon gene preservation. Furthermore, this approach enables us to easily control the freezing rate, which can be advantageous for the cryopreservation of embryonic cells. Typically, a slow freezing rate is required at the initial stage to minimize the formation of intracellular ice and facilitate vitrification (Gurruchaga et al., 2018).

The study revealed that post-thaw sperm motility and fertilization rates were lower than those of fresh sperm. The declines seen may be a result of reduced sperm viability percentage, increased sperm cell damage, or lowered ATP content after cryopreservation (Alavi et al., 2012). On the other hand, it should be known that the presence of a functional acrosome makes sturgeon and paddlefish spermatozoa unique compared to other fish sperm. Most studies on preserving chondrostean sperm through cryopreservation have established techniques that yield high motility but low rates of fertilization (Horvath et al., 2009). From this point of view, it is clear that structural changes in sperm cells are accountable for this, without affecting sperm motility. This is probably due to the presence of acrosomes, a characteristic that is lacking in the majority of teleosts. Psenicka et al. (2008) examined cryopreserved sterlet sperm to determine why DMSO produced high post-thaw motility but low fertilization rates in sturgeon sperm compared to methanol. According to their findings, the sperm motility features following cryopreservation with methanol or DMSO did

not differ significantly. However, specific acrosomal staining was 12.7% higher in sperm cryopreserved with DMSO than in sperm cryopreserved with methanol. According to these authors, the staining results indicated that DMSO caused damage to the acrosome during cryopreservation, resulting in lower fertility.

Conclusion

In conclusion, this study demonstrates that cryopreservation of Persian and stellate sturgeons' sperm is possible in a cryosolution with 15% DMSO and sucrose, using 0.50-ml straws. The applied protocol is ideal for commercial hatcheries supporting the artificial reproduction of Persian and stellate sturgeons, given the promising post-thaw motility and fertility results. This study has also shown that frozen sperm has a vital role in conserving the endangered sturgeon stock, despite having only 10% sperm motility after thawing. The creation of new techniques and the use of supplements can significantly improve the survival of frozen sperm and promote the establishment of cryobanks for *ex-situ* conservation of sturgeons.

Ethical Statement

Not Appicable.

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Author Contribution

Conceptualization: YB, Data Curation: MHS, Formal Analysis: MHS, Investigation: MHS, Methodology: MHS, Visualization: YB, Writing-original draft and Editing: YB

Conflict of Interest

The authors does not have any conflict of interest.

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