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Cryopreservation of Beluga (*Huso huso*) Sperm: Effect of Different Concentrations of DMSO and Dilution Rates on Sperm Mobility and Motility Duration After Short-Term Storage

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Abstract: Semen obtained from four Beluga males (*Husohuso*) was cryopreserved using extender; Tris-sucrose-KCl (30mM Tris, 23.4mM sucrose, 0.25mM KCl, pH 8.0) supplemented with DMSO atconcentration of 5%, 10% and 20%. Semen was diluted, respectively, with ratios of 1:0.5, 1:1 and 1:2 with extender and frozen in liquid nitrogen vapor.Frozen sperms after 3 and 15 days were excluded from freezing. Experiment showed the highest motility duration and the most motility percentage of post thawed sperms after 3 days was related to the treatments with the concentration of DMSO 10% and the dilution ratio of 1: 1 (336.12±27.52sand 30.31±3.94%; $P<0.05$), as well as the upmost mobility and the motility duration of post thawed sperms after 15 days was related to the treatments with the concentration of DMSO 10% and the dilution ratio of 1: 1 (253.32±21.52 s and 23.41±1.60%; $P<0.05$).

Key words: *Huso huso* · Cryopreservation · Sperm · DMSO · Motility duration · Motility percentage

INTRODUCTION

It has been estimated that semen from 200 fish species have been successfully cryopreserved [1]. However, species-specific optimizations of technology are needed. Main parameters for cryopreservation include kinds of extenders and cryoprotectants, the dilution rate, the freezing and thawing rates and kind of extender used for fertilization. The most efficient permeating cryoprotectant for fish semen appears to be dimethyl sulfide (DMSO) and methanol (MeOH) [2].

Other cryoprotectants such as glycerol, ethylene glycol, propane-diol, dimethyl acetamide and methanol are short popular or have been used with limited success. Unlike most teleost fish, information concerning reliable technology for cryopreservation of sturgeon milt is not available.

Cryopreservation success was usually measured as post-thaw sperm mobility [3, 4] or as fertilization success during primary embryo growth [5].

Sturgeon (*Acipenser sp., Chondrostei*) spermatozoa are significantly different from teleost fish sperm. These differences concern morphology (more complex structure, presence of acrosome), physiology (longer duration of mobility, acrosome reaction) and biochemistry (presence of acrosin,arylsulfatase) [6, 7]. Other striking difference between semen properties of sturgeons and teleost fish is the low osmolality of sturgeon seminal plasmacomposition [8]. The objectives of our work were to test the effect of: (1) DMSO in different concentrations on the motility percentage and motility duration of Beluga sturgeon sperm; (2) several dilution rates in combination with different DMSO concentrations on the motility percentage and motility duration of Beluga sturgeon sperm.

MATERIALS AND METHODS

Sperm Collection for Cryopreservation: Semen samples were collected from eight males of Beluga (*Huso huso*) in Shahid Marjani Sturgeon Hatchery located in Gorgan, Iran in March 2012. Spermiation was induced by injecting of sturgeon with pituitary extract in dose of 2-3 mg kg^{-1} body weight [9]. Semen was collected within 16-24 h (depending on the water temperature) post hormonal injection. Semen was transferred to Aquaculture

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Research Center of Gorgan University of Agricultural Sciences and Natural Resources. Milt was stored on ice and used within 2 h of storage forcryopreservation.

Assessment of Sperm Quality: Mobility of sperm samples was estimated under a light microscope at 400× magnification immediately after mixing of 5µL of sperm with 50µL of activation solution) NaCl3.5 mM, Tris-HCl12 mM, pH=8.5) [10] on a microscope slide. Sperm mobility and duration of sperm motility was recorded using a software gadmeitv home media v. 330 from note book connected to Nikon microscope (Optiphot-2, Japan) at 400× magnification that combined with CCD color video camera (model SPC-2000P, Japan). Sperm motility and duration of sperm motility were evaluated from sperm with forward movement. Immotile sperm were defined as sperm that did not show forwardmovement after activation. Video records were set at 30 frames/s using video camera mounted ona microscope. Percentage of sperm motility was determined during 0-10 s post-activation. Motilityduration was evaluated by counting the time from sperm activation with activation solution untilsperm stopped moving [11].

Extender and Sperm Cryopreservation: In these experiment using extender Tris- sucrose-KCl (30mM Tris, 23.4 mM sucrose, 0.25 mM KCl, pH 8.0) [12] were supplement with 5%, 10% and 20% DMSO[21]. Semen and extender had a temperature of 4°C. Milt was diluted at ratios of 1:0.5, 1:1 and 1:2with extender.

Suspensions of extended milt were drawn within 0.25-ml straws. Semen-freezing was conducted in a styrofoam box filled with liquid nitrogen. Straws were placed on a 4-cm-high floating frame made of styrofoam. Straws were sealed and after 3 min of freezing in liquidnitrogen vapor, were plunged within liquid nitrogen [2].

Measurement Sperm Mobility, Duration of Sperm Motility and Concentration: Straws were thawed in a water bath with a temperature of 40°C for 15 s [2]. Sperm mobility and duration of sperm motility of thawed semen was observed after 3 and 15 day of storage in liguid N2. Post-thaw mobility and motility duration was observed and evaluated bythe same operators using amonitor connected to a microscope.Semen concentration was measured by the LamNyvbarmethod [13].

Statistical Software: Microsoft Excel and SPSS version 16.0 were used for statistical analysis.

RESULTS

The duration of sperm ofsemen samples showing 80% motility or higher were used for the experiments (Table 1).

Effect of Dilution Rates with Concentrations of DMSO on Quality Post-Thawed Sperms after 3 Days: Highestmotility duration and the most motility percentage of post-thawed sperms after 3 dayswas related to the treatments with the concentration of DMSO 10% and the dilution of 1: 1(336.12±27.52 s and 30.31±3.94%; Table 2). The least Duration and the lowest mobility of post-thawed sperms was observed in the treatments with the concentration of DMSO 20% and the dilution of 1: 2. (260.67±24.48 s and 21.36±4.14%; $P<0.05$) Table 2.

Table 2 showed the maximum duration and the most mobility results observed intreatments where the dilution rate was 1:1, as well as the lowest motility percentage and motilityduration of post-thawedsperm was observed in dilution rate 1:2.

Effect of Dilution Rateswith Concentrations of DMSO on Quality Post-Thawed Sperms after 15 Days: Maximum motility duration and the upmost mobility of post-thawed sperms after 15 days wasrelated to the treatments with the concentration of DMSO 10% and the dilution of 1: 1 (253.32±21.52 s and 23.41±1.60%; Table 3).

Results shows the minimum Duration and the lowest motility percentage of post-thawed sperms in the treatments with the concentration of DMSO 20% and the dilution of 1: 2 (192.54±21.48 s and 15.13±1.95%; $P<0.05$).

Results showed the highest motility duration and the most motility percentage results in treatments where the dilution rate was 1:1, as well as minimum motility duration and the least mobility of post-thawed sperm was observed in dilution rate 1:2.

DISCUSSION

Decrease in stocks and limited number of potential breeders has led to the establishment offish sperm cryobanks which play a crucial role in the genetic management and conservation of aquatic resources [14, 15]. The establishment of semen banks from valuable fish species including sturgeon is widely practiced in multitplecountries [16, 17].

According to the above results, by comparing Table 2 and 3 the dilutionrates has significant differences on the duration of sperm motility ($P<0.05$), as most ofthe

Table 1: Males used for sperm cryopreservation process

Male	Body weight (g)	Total length (cm)	Sperm concentration ($\times 10^9 ml^{-1}$)	Motility duration (s)	Motility percentage (%)
1	170	291	2.30	430.21±12.14	82.32±2.41
2	160	240	2.56	410.64±8.75	83.43±2.70
3	150	260	2.02	418.52±10.42	82.46±1.87
4	135	260	2.87	407.34±9.61	84.32±2.04
Total	153.75	262.75	2.41	416.68±12.72	83.13±2.11

Table 2: Effect of different concentrations of DMSOand dilution rates on post-thaw sperm motility and duration of sperm motility after 3 days of freezing

Cryoprotectant	Cryoprotectant concentration (%)	Diluted rates (sperm: extender)	Motility duration (s)	Motility percentage (%)
		1 : 0.5	318.24±18.30 ^{a}	26.33±4.81 ^{ab}
DMSO	5	1 : 1	332.31±24.50 ^{a}	29.42±5.08 ^{a}
		1 : 2	267.00±27.58 ^{c}	23.34±3.10 ^{ab}
		1 : 0.5	323.24±18.81 ^{a}	27.20±4.80 ^{ab}
DMSO	10	1 : 1	336.12±27.52 ^{a}	30.31±3.94 ^{a}
		1 : 2	272.33±31.81 ^{bc}	24.54±4.10 ^{ab}
		1 : 0.5	312.24±22.30 ^{ab}	24.66±2.71 ^{ab}
DMSO	20	1 : 1	319.00±23.33 ^{a}	27.09±38.0 ^{ab}
		1 : 2	260.67±24.48 ^{c}	21.36±4.14 ^{b}
Control	-	-	416.68±12.72	83.13±2.11

Values within column followed by different superscript letters were significantly different (P<0.05)

Table 3: Effect of different concentrations of DMSOand dilution rates on post-thaw sperm motility and duration of sperm motility after 15 days of freezing

Cryoprotectant	Cryoprotectant concentration (%)	Diluted rates (sperm: extender)	Motility duration (s)	Motility percentage (%)
		1 : 0.5	225.21±25.73 ^{abc}	19.21±2.20 ^{bcd}
DMSO	5	1 : 1	249.33±22.53 ^{ab}	21.57±1.45 ^{ab}
		1 : 2	208.37±21.58 ^{bc}	16.37±1.60 ^{cde}
		1 : 0.5	226.65±26.37 ^{abc}	19.63±1.70 ^{bc}
DMSO	10	1 : 1	253.32±21.52 ^{a}	23.41±1.60 ^{a}
		1 : 2	213.67±20.81 ^{abc}	17.13±2.20 ^{cde}
		1 : 0.5	221.00±22.30 ^{abc}	16.11±1.21 ^{de}
DMSO	20	1 : 1	230.47±19.33 ^{abc}	18.36±1.60 ^{bcde}
		1 : 2	192.54±21.48 ^{c}	15.13±1.95 ^{e}
Control	-	-	416.68±12.72	83.13±2.11

Values within column followed by different superscript letters were significantly different (P<0.05)

motility duration related to dilution ratio of 1: 1of the treatments significantly reduced with increasing dilution. Because of the high dilution of the sperm, plasma loses its protective effect, sperm viability reducedand, the concentration of cryoprotectantin increased causing toxicity resulting in reduced sperm viability [18]. The results showed that thesperm quality significantly reduced after thawing similar to the resultsof [19]. Theseresearchers have reported that the quality of Ponto-Caspian sturgeonsemen sharplydecreased after thawing. In this experiment, post-thawed sperms with DMSO concentration of 10% and the dilution of 1:1 has the highest mobility and motility duration similar to the results of [20]. These researchers have reported that themost suitable cryoprotectant for sperm cryopreservation Italian Cobice sturgeon (*Acipenser naccarii*), is DMSO concentration of 10%. Also, [18]announced the mostmotility of post-thawed sperm Starlet (*Acipenser ruthenus*) was with DMSO 10% (80±7.4%).

[21] announced the maximum motility and motility duration of post-thawed sperm of pallid sturgeon (*Scaphyrinchusalbus*) was with DMSO concentration of 5% (26±13 %). [22] reported that themost suitable cryoprotectant forsperm Cryopreservation Chinese sturgeon (*Acipenser sinensis*), was with DMSO 12%.

The reason of difference in suitable density of cryoprotectant in written result with experiment result may be for selected species, difference in extender solution or semen isspecific characteristics of this species.

CONCLUSION

From the above study it can be concluded that storage of frozen sperm has a negative impact on motility duration and motility percentage of post-thawed sperms. Results also showed that highest motility duration and motility percentage of post-thawed sperms was for treatments with the concentration of DMSO 10% and the dilution of 1:1.

REFERENCES

1. Billard, R., J. Cosson, L.W. Crim and M. Suquet, 1995. Sperm physiology and quality. In: N.R. Bromage and R.J. Roberts, (Eds.), Broodstock Management and Egg and Larval Quality. Blackwell, Oxford, pp: 25-52.
2. Glogowski, J., R. Kolman, M. Szcepkowski, A. Horvath, B. Urbanyi, P. Sieczynski, A. Rzemieniecki, J. Domagala, W. Demianowicz, A. Kowalski and A. Ciereszko, 2002. Fertilization rate of Siberian sturgeon (*Acipenser baerii*) milt cryopreserved with methanol. Aquaculture, 211: 367-373.
3. Ciereszko, A., G.P. Toth, S.A. Christ and K. Dabrowski, 1996. Effect of cryopreservation and theophylline on motility characteristics of lake sturgeon (*Acipenser fulvescens*) spermatozoa. Theriogenology, 45: 665-672.
4. Billard, R., J. Cosson and O. Linhart, 2000. Changes in the flagellum morphology of intact and frozen/thawed Siberian sturgeon *Acipenser baerii* (Brandt) sperm during motility. Aquaculture Reserch, 31: 283-287.
5. Tsvetkova, K.I., J. Cosson, O. Linhart and R. Billard, 1996. Motility and fertilizing capacity of fresh and frozen-thawed spermatozoa in sturgeon, *Acipenser baerii* and *Acipenser ruthenus*. Journal of Applied Ichthyology, 12: 107-112.
6. Dettlaff, T.A., A.S. Ginsburg and O.I. Schmalchausen, 1993. Sturgeon fishes. Developmental Biology and Aquaculture. Springer-Verlag, Berlin, pp: 67-71.
7. Ciereszko, A., K. Dabrowski, F. Lin and S.I. Doroshov, 1994. Identification of trypsin-like activity in sturgeon spermatozoa. Theriogenology, 268: 486-491.
8. Gallis, J.L., E. Fedrigo, P. Jatteau, E. Bonpunt and R. Billard, 1991. Siberian sturgeon *Acipenserbaeri* spermatozoa; effects of dilution, pH, osmotic pressure, sodium and potassium on motility. In: P. Willot, (Ed.), Acipenser. Cemagref Publ., Bordeaux, France, pp: 143-151.
9. Williot, P., E.F. Kopeika and B.F. Goncharov, 2000. Influence of testis state, temperature and delay in semen collection on spermatozoa motility in the cultured Siberian sturgeon (*Acipenserbaeri* Brandt). Aquaculture, 189: 53-61.
10. Jahnichen, H., W. Warnecke, E. Trolsch, K. Kohlmann, H. Bergler and H.J. Pluta, 1999. Motility and fertilizing capability of cryopreserved *Acipenserruthenis*L. sperm. Journal of Applied Ichthyology, 15: 204-206.
11. Irawan, H., V. Vuthiphandchai and S. Nimrat, 2010. The effect of extenders, Animal cryoprotectants and cryopreservation method on common carp (*Cyprinuscarpio*) sperm.Animal Reproduction Science, 122: 236-243.
12. Urbanyi, B., A. Horvath and M. Bercsenyi, 2000. Androgenesis on sterlet (*Acipenser ruthenus*) using fresh and cryopreserved sperm. 6th International Symposium on Reproductive Physiology of Fish, Bergen, Norvegia, 1999, julius 4-9. (Proceedings P. 440 Eds: B. Norberg, O.S. Kjesbu, G.L. Taranger, E. Andersson and S.O. Stefansson, Bergen).
13. Ciereszko, A. and K. Dabrowski, 1993. Estimation of sperm concentration of rainbow trout, whitefish and yellow perch using spectrophotometric technique. Aquaculture, 109: 1292-1305.
14. Linhart, O., R. Billardand J.P. Proteau, 1993. Cryopreservation of European catfish (*Silurusglanis* L.) spermatozoa. Aquaculture, 115: 347-359.
15. Billard, R. and T. Zhang, 2001. Techniques of genetic resource banking of fish. In: P.F. Watson and W.V. Holt (eds). Cry banking the genetic resource, wildlife conservation for the future. Taylor and Francis, London, UK, pp: 144-170.
16. Kopeika, E.F., P. Williot and B.F. Goncharov, 2000. Cryopreservation of Atlantic sturgeon, *Acipensersturio* L., 1758 sperm: First results and associated problems. Bulletin of the Institute of Spanish Oceanography, 16: 167-173.
17. Chao, N.H. and H.C. Liao, 2001. Cryopreservation of finfish and shellfish gametes and embryos. Aquaculture, 197: 161-189.
18. Lahnsteiner, F., B. Berger, A. Horvath and B. Urbanyi, 2004. Stuies on the semen biology and sperm cryopreservation in the starlet, (*Acipenser ruthenus*). Aquaculture Res., 35: 519-528.
19. Dzuba, B.B., F.F. Kopeika, V.V. Cherepanov and S.I. Drokin, 1999. Sturgeon sperm quality after 6 years of cryopreservation. Journal of Applied Ichthyology, 15: 312-318.

20. Galli, A., R. Vanni, S. Rossetti and R. Aleandri, 2006. Milt cryopreservation in Italian Cobice sturgeon *Acipenser naccarii*. The Aquaculture Society, pp: 272.
21. Horvath, A., W.R. Wayman, B. Urbanyi, K.M. Ware, J.C. Dean and T.S. Tiersch, 2005. The relationship of the cryoprotectants methanol and dimethyl sulfoxide and hyperosmoticextenders on sperm cryopreservation of two North-American sturgeon species. Aquaculture, 247: 243-251.
22. Liu, L., Q. Wei, F. Guo and T. Zhang, 2006. Cryopreservation of Chinese sturgeon (*Acipenser sinensis*) sperm. Journal of Applied Ichthyology, 22: 384-388.