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Effect of Different Concentrations of DMSO and Dilution Rates on Viability of Beluga (*Huso huso*) Post-Thawed Sperm

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Abstract: Milt obtained from four Beluga males (*Huso huso*) was cryopreserved using extender; Tris-sucrose-KCl (30mM Tris, 23.4mM sucrose, 0.25mM KCl, PH 8.0) supplemented with DMSO at concentration 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 30 and 60 days were excluded from freezing. Experiment showed the highest motility duration and the most motility percentage of post-thawed sperms after 30 days was related to the treatments with the concentration ofDMSO 10% and the dilution ratio of 1: 1 (129±24.52s and12.63±4.52%; $P<0.05$), as well as the upmost mobility and the motility duration of post-thawed sperms after 60 days was related to the treatments with the concentration of DMSO 10% and the dilution ratio of 1: 1 (90.33±22.52 s and10.08±4.32%; $P<0.05$). The results showed that holding time from 30 to 60 days 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 that have been diluted with 1:1 ratio.

Key words: *Huso huso* · Cryopreservation · Sperm · DMSO · Motility Duration · Motility Percentage

INTRODUCTION

Sturgeons (Order Acipenseriformes) are chondrostean fishes of classical origin that inhabit only the Northern hemisphere [1]. Several species are restricted to very little populations which in some cases are close to extinction due to exploitation of natural stocks for meat and caviar as well as destruction of habitat [2]. It has been estimated that semen from 200 fish species have been successfully cryopreserved [3]; however, species-specific optimizations of technology are needed. Successfully cry opreservation of fish spermatozoa is well established for many species and dimethyl sulfoxide is the maximum commonly used cryoprotectant [4]. Earlier works indicated that the cryopreservation of sturgeon spermatozoa using DMSO-sucrose extender resulted in improvement of motile spermatozoa with basic motility characteristics alike to those of fresh semen [5]. Main parameters for cryopreservation include types of extenders and cryoprotectants, the dilution ratio, 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) [6]. Other cryoprotectants such as glycerol, ethylene glycol, propane-diol, dimethyl acetamide and methanol are short popular or have been used with limited success. Unlike maximum teleost fish, information concerning reliable technology for cryopreservation of sturgeon semen is not available. Cryopreservation success was usually measured as post-thaw sperm mobility [5, 7] or as fertilization success during primary embryo growth [8]. Sturgeon (*Acipenser sp., Chondrostei*) semen 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) [9, 10].Other striking diversity between milt

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properties of sturgeons and teleost fish is the low osmolality of sturgeon seminal plasma composition [11]. 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

Semen Collection for Cryopreservation: Semen samples were collected from four males of Beluga (*Huso huso*)in Shahid Marjani Sturgeon Hatchery located in Gorgan, Iran in March 2012. Spermiation was induced by injecting of sturgeon pituitary extract in dose of 2-3mg kg^{-1} body weight [12]. Spermatozoa were collected within 16-24h (depending on the water temperature) post hormonal injection. Semen was transferred to Aquaculture Research Center of Gorgan University of Agricultural Sciences and Natural Resources. Milt was stored on ice and used within 2 h of storage for cryopreservation.

Assessment of Sperm Quality: Mobility of semen samples was estimated under a light microscope at 400× magnification immediately after mixing of 5µL of sperm with 50µL of activation solution) NaCl 3.5 mM, Tris-HCl 12 mM, pH=8.5) [13]on a microscope slide. Sperm motility percentage and duration of sperm motility was recorded using a software gadmei tv 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 mobility and duration of sperm motility were evaluated from sperm with forward movement. Immotile sperm were defined as sperm that did not show forward movement after activation. Video records were set at 30 frames/s using video camera mounted on a microscope. Percentage of sperm motility was determined during 0–10 s post-activation. Motility duration was evaluated by counting the time from sperm activation with activation solution until sperm stopped moving [14].

Extender and Sperm Cryopreservation: In these tests using extender Tris- sucrose-KCl(30mM Tris, 23.4mM sucrose, 0.25mMKCl, PH 8.0)[15] were supplement with 5%, 10% and 20% DMSO [16]. Sperm 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 into 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 not sealed and after 3 min of freezing in liquid nitrogen vapor, were plunged within liquid nitrogen [6].

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 [6]. Sperm mobility and duration of sperm motility of thawed sperm was observed after 30 and 60 day of storage in liguid N2. Post-thaw mobility and motility duration was observed and evaluated by the same operators using a monitor connected to a microscope. Semen concentration was measured by the LamNyvbarmethod [17].

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

RESULTS

The duration of motility of semen used for cryopreservation exceeded 400 s (Table 1). Likewise, only sperm samples showing 80% mobility or higher were used for the experiments (Table 1).

Effect of dilution rates with concentrations of DMSO on quality post-thawed sperms after 30 days.

Highest motility duration and the most motility percentage of post-thawed sperms after 30 days was related to the treatments with the concentration of DMSO 10% and the dilution of 1: 1 (129±24.52 s and 12.63±4.52%; Table 2). The least duration and the lowest mobility of post-thawed sperms was observed in the treatments with the concentration of DMSO 5% and the dilution of1: 0.5. (71.67±21.73 s and 5.61±2.15%; $P<0.05$) Table 2.Results showed the maximum duration and the most mobility results observed in treatments where the dilution rate was 1:1, as well as the lowest motility percentage and motility duration of post-thawed sperm was observed in dilution rate 1:0.5.

Effect of Dilution Rates with Concentrations of DMSO on Quality Post-Thawed Sperms after 60 days: Maximum motility duration and the upmost mobility of post-thawed sperms after 60 days was related to the treatments with the concentration of DMSO 10% and the dilution of 1: 1 (90.33±22.52 s and 10.08±4.32%; Table 3). Results shows the minimum duration and the lowest motility percentage of post-thawed sperms in the treatments with the concentration of DMSO 5% and the dilution of 1: 0.5 (41.84±23.57 s and 3.24±2.73%; $P<0.05$).

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±24.14	82.32±2.41
2	160	240	2.56	410.64±18.75	83.43±2.70
3	150	260	2.02	418.52±20.42	82.46±1.87
4	135	260	2.87	407.34±22.61	84.32±2.04
Total	153.75	262.75	2.41	416.61±20.49	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 30 days of freezing

Cryoprotectant	Cryoprotectant concentration (%)	Diluted rates (sperm: extender)	Motility duration (s)	Motility percentage (%)
		1: 0.5	71.67±21.73^{b}	5.61 ±.2.15^{a}
DMSO	5	1: 1	126.31 ±22.27^{a}	13.34 ±4.08^{a}
		1: 2	90.43 ±24.58^{ab}	11.00 ±.3.80^{a}
		1: 0.5	73.33 ±24.16^{b}	8.47 ±5.52^{a}
DMSO	10	1: 1	129.00 ±24.52^{a}	12.63 ± 4.52^{a}
		1: 2	114.48 ±20.81^{ab}	11.36 ±5.10^{a}
		1: 0.5	88.62 ±20.31^{ab}	8.00 ±4.28^{a}
DMSO	20	1: 1	120.67 ±27.22^{a}	10.20 ±2.15^{a}
		1: 2	102.30 ±22.48^{ab}	9.14 ±4.08^{a}
Control	-	-	416.61±20.49	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 60 days of freezing

Cryoprotectant	Cryoprotectant concentration (%)	Diluted rates (sperm: extender)	Motility duration (s)	Motility percentage (%)
		1: 0.5	41.84±23.57^{b}	3.24±2.73^{b}
DMSO	5	1: 1	84.00 ±22.53^{ab}	8.31 ±3.10^{ab}
		1: 2	71.23 ±21.58^{ab}	7.24 ±.2.83^{ab}
		1: 0.5	58.24 ±20.50^{ab}	6.24 ±4.52^{ab}
DMSO	10	1: 1	90.33 ±22.52^{a}	10.08 ± 4.32^{a}
		1: 2	78.00 ±20.81^{ab}	8.62 ±4.24^{ab}
		1: 0.5	70.63 ±22.30^{ab}	6.67 ±2.40^{ab}
DMSO	20	1: 1	80.52 ±18.70^{ab}	7.12 ±3.60^{ab}
		1: 2	74.10 ±21.48^{ab}	5.94 ±1.52^{ab}
Control	-	-	416.61±20.49	83.13±2.11

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

Table 3 showed the highest motility duration and the most motility percentage results observed 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 1:0.5.

DISCUSSION

The cryopreservation remains one of the most attractive and quickly developing trend for the sturgeon protection. Methods of cryopreservation of the sturgeon semen have been well established[6, 18]. However, the different steps required for cryopreservation (cryoprotective agent loading, freezing/thawing, cooling to a low subzero temperature) may contribute individually or cumulatively to semen damage that in turn decreases fertilization and growth stages [19]. Recently, it has been shown, that profound freezing mechanically destroys cell membranes [20].

According to the above results, by comparing Table 2 and 3 the dilution rates has significant differences on the duration of sperm motility (P<0.05), as most of the motility duration related to dilution ratio of 1: 1 of the treatments and significantly reduced with increasing dilution. Because of the high dilution of the sperm, plasma loses its protective effect, sperm viability reduced and the concentration of cryoprotectant increased causing toxicity resulting in reduced sperm viability [21]. The results showed that the sperm quality significantly reduced after thawing similar to the results [22]. These researchers have reported that the quality of Ponto-Caspian sturgeon semen sharply decreased 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 [23]. These researchers have reported that the most suitable cryoprotectant for sperm cryopreservation Italian Cobice sturgeon (*Acipenser naccarii*), is DMSO

concentration of 10%. Also, Lahnsteiner *et al*. [21] announced the most motility of post-thawed sperm Starlet (*Acipenser ruthenus*) was with DMSO 10% (80±7.4%). In this experiment, post-thawed sperms with DMSO concentration of 5% has the lowest mobility and motility duration and this was in contrast to the results obtained by the [16]. These reserchers have Announced that the maximum motility and motility duration of post-thawed sperm of pallid sturgeon (*Scaphyrinchus albus*) was with DMSO concentration of 5% (26±13 %) Liu *et al.* [24] reported that the most suitable cryoprotectant for sperm Cryopreservation Chinese sturgeon (*Acipenser persicus*), 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 is specific 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. Birstein, B.J. and R. DeSalle, 1998. Molecular phylogeny of Acipenserinae. Mol. Phylogenet. Evol., 9: 141-155.
2. 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.
3. 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.
4. Leung, L.K.P. and B.G.M. Jamieson, 2000. Live preservation of fish gametes, in: B.G.M. Jamieson (Ed.), Fish Evolution and Systematics: Evidence from Spermatozoa, Cambridge University Press, Cambridge, London, UK, pp: 245-269.
5. 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.
6. 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 baeri) milt cryopreserved with methanol. Aquaculture, 211: 367-373.
7. 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.
8. Tsvetkova, K.I., J. Cosson, O. Linhart and R. Billard, 1996. Motility and fertilizing capacity of fresh and frozen-thawed spermatozoa in sturgeon, Acipenser baeriand and *Acipenser ruthenus*. Journal of Applied Ichthyology, 12: 107-112.
9. Dettlaff, T.A., A.S. Ginsburg and O.I. Schmalchausen, 1993. Sturgeon fishes. Developmental Biology and Aquaculture. Springer-Verlag, Berlin, pp: 67-71.
10. Ciereszko, A., K. Dabrowski, F. Lin and S.I. Doroshov, 1994. Identification of trypsin-like activity in sturgeon spermatozoa. Theriogenology, 268: 486- 491.
11. Gallis, J.L., E. Fedrigo, P. Jatteau, E. Bonpunt and R. Billard, 1991. Siberian sturgeon Acipenser baeri spermatozoa; effects of dilution, pH, osmotic pressure, sodium and potassium on motility. In: P. Willot, (Ed.), Acipenser. Cemagref Publ., Bordeaux, France, pp: 143-151.
12. 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 (Acipenser baeri Brandt). Aquaculture, 189: 53-61.
13. Jahnichen, H., W. Warnecke, E. Trolsch, K. Kohlmann, H. Bergler and H.J. Pluta, 1999. Motility and fertilizing capability of cryopreserved Acipenser ruthenis L. sperm. Journal of Applied Ichthyology, 15: 204-206.
14. Irawan, H., V. Vuthiphandchai and S. Nimrat, 2010. The effect of extenders, Animal cryoprotectants and cryopreservation method on common carp (Cyprinus carpio) sperm. Animal Reproduction Science, 122: 236-243.

15. 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 pp: 440 Eds: B. Norberg, O.S. Kjesbu, G.L. Taranger, E. Andersson and S.O. Stefansson, Bergen).
16. 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 hyperosmotic extenders on sperm cryopreservation of two North-American sturgeon species. Aquaculture, 247: 243-251.
17. Ciereszko, A. and K. Dabrowski, 1993. Estimation of sperm concentration of rainbow trout, whitefish and yellow perch using spectrophotometric technique. Aquaculture, 109: 1292-1305.
18. Drokin, S.I., V.V. Cherepanov, E.F. Kopeika and N.I. Shilin, 1993. Cryopreservation of the sperm of (Acipenser mikadoi) problems and prospects for cryopreseved collection from rare and endangered sturgeon species. Int. Symp. Sturgeons M-K-M-. VNIRO, Moscow, pp: 64-65.
19. Friedler, S., L.C. Giudice and E.J. Lamb, 1988. Cryopreservation of embrios and ova, FertilSteril, 49: 743-764.
20. Parks, J.E. and J.K. Graham, 1992. Effects of cryopreservation procedures on sperm membranes, Theriogenology, 38: 209-222.
21. Lahnsteiner, F., B. Berger, A. Horvath and B. Urbanyi, 2004. Stuies on the semen biology and sperm cryopreservation in the starlet, (Acipenser ruthenus). Aquaculture Reserch, 35: 519-528.
22. 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.
23. Galli, A., R. Vanni, S. Rossetti and R. Aleandri, 2006. Milt cryopreservation in Italian Cobice sturgeon Acipenser naccarii. The Aquaculture Society, pp: 272.
24. Liu, L., Q. Wei, F. Guo and T. Zhang, 2006. Cryopreservation of Chinese sturgeon (Acipenser sinensis) sperm. Journal of Applied Ichthyology, 22: 384-388.