STUDY REGARDING SHORT TIME PRESERVATION OF SPERM FROM *POLYODON SPATHULA* BREED

C.-A. Barbacariu[1*], M.C. Cioran[1], B. Păsărin[1]

[1]*University of Agricultural Sciences and Veterinary Medicine from Iaşi, Romania*

Abstract

The aim of the current paper was to determine a preservation protocol of sperm from Polyodon spathula on a short period of time at a constant temperature of 4ºC, using three preservation methods for optimizing the process of artificial reproduction. Sperm was gathered from Polyodon spathula males, sexually mature with the age of 14 years which were reared in a fishery farm from Iaşi County, Romania. Males were hormonal stimulated by inter-muscular injection with a Russian hormone, Nerestin 5A. Sperm was gathered utilising milking method, by massaging their abdomens. After gathering was examined, to determine mobility using Persov method and was preserved by using three methods. After storage sperm from all those three batches was analysed after 2, 18, 36 and 48 hours, determining the mobility of spermatozoa. The most efficient preservation method was the one in which sperm was stored in nylon bags, sterile, in which technical oxygen was injected. Aren't great differences between the sperm kept in air bag in comparison with the one with addition of technical oxygen, both methods leading to a longer preservation of sperm mobility.

Key words: *Polyodon spathula,* sperm, short time preservation, sturgeons

INTRODUCTION

Together with the acclimatization of *Polyodon spathula* breed in Romania, was necessary to be developed the studies regarding the technology of artificial reproduction, for an easier access of it at the level of fishery farms. High growing rate of the specie, feeding specific (mainly plankton) and the quality of produced caviar made that those one to be a very important rearing specie [5].

Studies regarding physiology of sperm as well as its mobility offer us the basic knowledge to reach the success in fishes' artificial reproduction [13]. Evaluation of mobility of sperm gathered from fishes was studied in the last years by many researchers: [1], [3], [6], [9], [15]. Knowledge of relations between sperms' characteristics (density and pH) and fertilization capacity allow the development of its storage capacity on short term [7]. Studies regarding short term preservation capacity of sperm gathered from fish were realised on many species like as: *Largemouth Bass* [8], *Polyodon spathula* [2], *Stizostedion viteum* [12], *Acipenser persicus* [10] salmonids [11] and others.

*Corresponding author: alin.barbacariu@yahoo.com
The manuscript was received: 03.09.2016
Accepted for publication: 17.10.2016

MATERIAL AND METHOD

The studied biological material was represented by 49 *Polyodon spathula* reproductive individuals from which were selected 24 males divided in three batches. All reproductive individuals were reared in the same environmental conditions at Farm for Production and Fishery Research SC Acvares SRL, Ţigănaşi, Iaşi. In each year, reproductive individuals were reared in pond with 30 ha and during winter were moved into a basin with an area of 1 ha.

At the beginning of March when water reached the temperature of 8ºC, reproductive individuals were separated on sexes and moved inside artificial reproduction station. When water temperature from inside artificial reproduction station reached the value of 14.5ºC, males were inner-muscular injected with a Russian origin hormone, Nerestin 5A. The utilised dose was of 0.1 ml/kg male body.

After 24 hours from injection sperm was gathered using milking method, by massaging of their abdomen or by introducing of a hose connected to a syringe. The sperm volume gathered from each male was different and was between the values of 2-8 ml/kg male body. After gathering sperm was examined, determining its mobility and being preserved

through three methods. Sperm was analysed using a MC 7 microscope with the lens focusing possibility of 10X, 20X, 40X.

Evaluation of spermatozoids' mobility was realised in conformity with Persov method [14]. In according with this method spermatozoids' mobility could be divided as follows:

- 5 points. Movement is rapid and forward for all the observed spermatozoids.
- 4 points. Movement is rapid and forward for almost all the observed spermatozoids but are also observed some of them which have zigzag and oscillating moves.
- 3 points. Movement is rapid and forward for some of spermatozoids, but predominantly is zigzag and oscillating move. Could be observed some immobile spermatozoids.
- 2 points. Rapid and forward movement is almost absent. Could be observed a low oscillating movement. Immobile spermatozoids are mostly predominant (more than 75%).
- 1 point. All spermatozoids are immobile.

This is one of the most utilised methods for evaluation of mobility of spermatozoids gathered from sturgeons' reproductive individuals [4].

To study the mobility, sperm samples were diluted in a rate of 1:20 and examined at room temperature, similar with the one were ejaculation took place.

Samples' preservation was made using three methods:

- first method consisted in storage of sperm into a plastic syringe with 20 ml capacity. In it were putted 10 ml of sperm gathered from one male, and in the rest of syringes' volume was introduced air.
- second method consisted in sperm storage into a nylon bag, sterile, hermetically closed with a volume of 2000 ml. In it were putted 10 ml of sperm gathered from one male, and in the rest of bags' volume was introduced technical oxygen.
- third method consisted in sperm storage into a nylon bag, sterile, hermetically closed with a volume of 2000 ml. In it were putted 10 ml of sperm gathered from one male, and in the rest of bags' volume was introduced air.

All samples from all those three batches were stored at a constant temperature of 4ºC. After storage sperm from all those three batches were analysed after 2, 18, 36 and 48 hours, determining the spermatozoids mobility by using the same method.

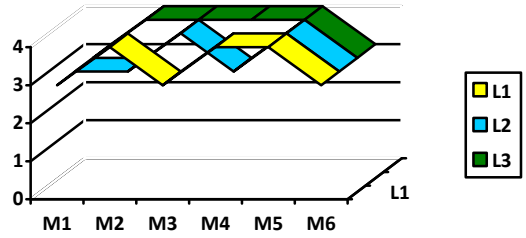
STATISTICAL SOFTWARE: Microsoft Excel and SPSS version 16.0 were used for statistical analysis.

RESULTS AND DISCUSSIONS

Immediately after gathering, sperm from all those three batches was examined and we determine the mobility of spermatozoids, in according with Persov method. The preliminary results are shown in table 1.

Table 1 Preliminary results for analysis of *Polyodon spathula* sperm

Specification	L1						L2						L3					
Males' number	M1	M2	M3	M4	M5	M6	M1	M2	M3	M4	M5	M6	M1	M2	M3	M4	M5	M6
Mobility points	3	4	3	4	4	3	3	3	4	3	4	4	3	4	4	4	4	3
X	21						21						22					
SX	0.55						0.55						0.52					
Min (%)	3						3						3					
Max (%)	4						4						4					
V (%)	15.65						15.65						14.08					
Significance of differences between batches	L1 vs. L2 vs. L3 = n.s.; F(0.1923) > Fα(3.6823), 2:15 df																	

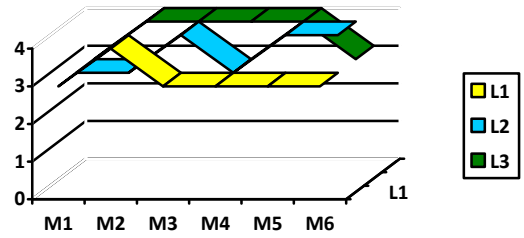


It could be observed that spermatozoids' mobility from all those three batches is around the value of 3-4 points associated to Persov scale, none of them having all those 5 points. That low mobility of spermatozoids is due to the fact that the whole batch is at the beginning of reproduction period accumulated degrees-hours by each reproductive individual being minimal for starting of spermation. From statistical point of view between mobility points associated to those three batches the differences were insignificant.

After 2 hours from gathering, sperm from the males of all those three batches were analysed. The results are presented in table 2.

Table 2 Results for evaluation of spermatozoids mobility after two hours from gathering

Specification	L1						L2						L3					
Males' number	M1	M2	M3	M4	M5	M6	M1	M2	M3	M4	M5	M6	M1	M2	M3	M4	M5	M6
Mobility points	3	4	3	3	3	3	3	3	4	3	4	4	3	4	4	4	4	3
X	3.17						3.35						3.67					
SX	0.41						0.55						0.52					
Min (%)	3						3						3					
Max (%)	4						4						4					
V (%)	12.59						15.65						14.08					
Significance of differences between batches	L1 vs. L2 vs. L3 = n.s.; F(1.5909) > Fα(3.6823), 2:15 df																	

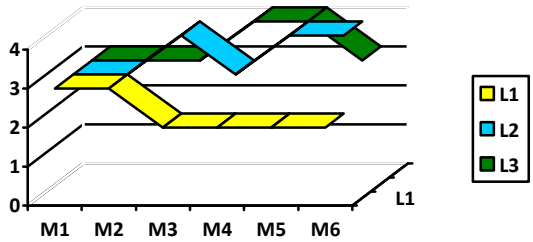


It could be observed a slow decrease of the points associated with Persov scale for all those three batches, a little bit more intense in the case of first batch where sperm was kept into a plastic syringe. From statistical point of view between mobility points associated to those three batches the differences were insignificant.

After 18 hours from gathering, sperm from the males of all those three batches were analysed. The results are presented in table 3.

Table 3 Results for evaluation of spermatozoids mobility after 18 hours from gathering

Specification	L1						L2						L3					
Males' number	M1	M2	M3	M4	M5	M6	M1	M2	M3	M4	M5	M6	M1	M2	M3	M4	M5	M6
Mobility points	3	3	2	2	2	2	3	3	4	3	4	4	3	3	3	4	4	3
X	2.33						3.5						3.33					
SX	0.52						0.55						0.52					
Min (%)	2						3						3					
Max (%)	3						4						4					
V (%)	22.13						15.65						15.49					
Significance of differences between batches	L1 vs. L2 vs. L3 = **.; F(8.6000) > Fα(3.6823), 2:15 df																	

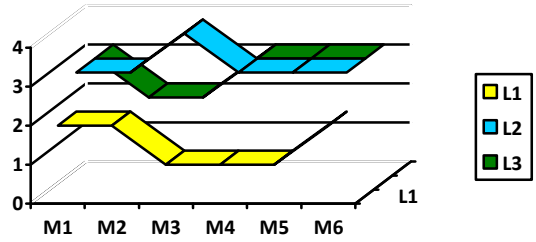


The decreasing of the points associated with Persov scale continued for the first batch where sperm was preserved into a plastic syringe but it is relatively constant for batches 2 and 3. From statistical point of view between mobility points associated to those three batches the differences were significant.

After 36 hours from gathering, sperm from the males of all those three batches were analysed. The results are presented in table 4.

Table 4 Results for evaluation of spermatozoids mobility after 36 hours from gathering

Specification	L1						L2						L3					
Males' number	M1	M2	M3	M4	M5	M6	M1	M2	M3	M4	M5	M6	M1	M2	M3	M4	M5	M6
Mobility points	2	2	1	1	1	2	3	3	4	3	3	3	3	2	2	3	3	3
X	1.5						3.17						2.67					
SX	0.55						0.41						0.52					
Min (%)	1						3						2					
Max (%)	2						4						3					
V (%)	36.51						12.89						19.36					
Significance of differences between batches	L1 vs. L2 vs. L3 = ** ; F(17.9545) > Fα(3.6823), 2:15 df																	

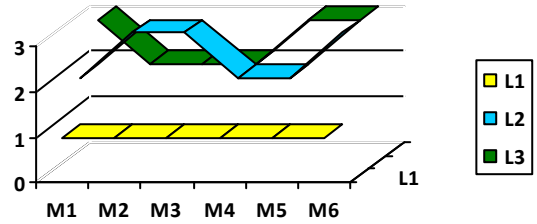


Same as after 18 hours continue the decreasing of points associated with Person scale for the first batch where sperm was kept into a plastic syringe but it is relatively constant for batches 2 and 3. From statistical point of view between mobility points associated to those three batches the differences were significant.

After 48 hours from gathering, sperm from the males of all those three batches were analysed. The results are presented in table 5.

Table 5 Results for evaluation of spermatozoids mobility after 48 hours from gathering

Specification	L1						L2						L3					
Males' number	M1	M2	M3	M4	M5	M6	M1	M2	M3	M4	M5	M6	M1	M2	M3	M4	M5	M6
Mobility points	1	1	1	1	1	1	2	3	3	2	2	3	3	2	2	2	3	3
X	1						2.5						2.5					
SX	0						0.55						0.55					
Min (%)	1						2						2					
Max (%)	1						3						3					
V (%)	0						21.91						21.91					
Significance of differences between batches' means	L1 vs. L2 vs. L3 = **; F(22.5000) > Fα(3.6823), 2:15 df.																	



Sperm associated with the males from first batch became immobile, the points from Persov scale having the value 1. For bathes 2 and 3 the mean of points associated with Presov scale was 2.5, which correspond to a rapid and forward move of some spermatozoids but immobile spermatozoids are predominant. Technologically, if sperm from a male had the value associate with Persov scale under 3 points couldn't be utilised at artificial reproduction. From statistical point of view between mobility

points associated to those three batches the differences were significant. Differences between batch 2 where sperm was preserved into a sterile bag and in which was introduced also oxygen and batch 3 where sperm was preserved into a sterile bag in which was introduced air were insignificant.

CONCLUSIONS

After initial analysis of sperm gathered from *Polyodon spathula* males it could be observed that spermatozoids mobility for all those three batches were around the value of 3-4 points associated with Persov scale, none of them having all those 5 points.

After two hours from preserving weren't recorded differences between those three preservation methods. After 18 hours from preservation it could be observed that sperm from the plastic syringe had a qualitative decreasing, being recorded a mean value of 2.33 points on Persov scale so it isn't suitable for artificial reproduction, but quality of sperm from those two bags remained constant. After 36 hours from preservation, mean value of sperm from plastic syringe reached at 1.5 points on Persov scale. The values of mobility points on Persov scale for the sperm in bags remain almost the same.

After 48 hours from preserving also the value of mobility points for the sperm in bags decreased so also these one being not suitable to be utilised.

The best method for short-time preservation of *Polyodon spathula* sperm was the one in sterile bag with addition of technical oxygen. Aren't great differences between the sperm kept in air bag in comparison with the one with addition of technical oxygen, both methods leading to a longer preservation of sperm mobility.

REFERENCES

[1] Bobe J., Labbé C., 2010: Egg and sperm quality in fish. Gen. Comp. Endocrinol., 165, 3, 535-548

[2] Brown G.G., Mims S.D., 1995: Storage, transportation, and fertility of undiluted and diluted paddlefish milt. Progressive Fish-Culturist 57:64*69.

[3] Cabrita E., Robles V., Herraez P., 2009: Sperm quality assessment. Methods in reproductive aquaculture. Marine and freshwater species. CRC Press Taylor & Francis Group, Boca Raton, USA, ISBN 978-0-8493-8053-2.

[4] Chebanov S.M., Galich V.E., 2013: Sturgeon hatchery manual, Fisheries and Aquaculture tehnical paper, FAO, Krasnodar, Russia

[5] Costache Mioara, Daniela Radu, Oprea D., Costache M., Nicolae Carmen Georgeta, 2012: Research considering spermiation on *Polyodon spathula* sturgeon species towards optimizing spawning biotechnology and conserving biodiversity, Lucrări Ştiinţifice seria Zootehnie, vol. 58, Iaşi, p. 246-250.

[6] Cosson J., Groison A.L., Suquet M., Fauvel C., Dreanno C., Billard R., 2008: Studying sperm motility in marine fish: an overview on the state of the art. J. Appl. Ichthyol. 24, 4, 460-486.

[7] Dilauro M.N., Krise W.F, Hendrix M.A., Baker S.E., 1994: Short-term cold storage of Atlantic sturgeon sperm. Progressive Fish-Culturist 56: 143-144.

[8] Erdahl A.W., Erdahl D.A., Graham E.E., 1984: Some factors affecting the preservation of salmonid spermatozoa. Aquaculture 43:341-35.

[9] Fauvel C., Suquet M., Cosson J., 2010, Evaluation of fish sperm quality, Journal of Applied Ichthyology Volume 26, Issue 5, pages 636–643

[10] Hadiseh Dadras, Hosein Khara, Shahrouz Baradaran Noveiri, 2013: Effect of sperm pH and density on fertilization success in Persian sturgeon *Acipenser persicus* (Borodin, 1897), Springer-Verlag London.

[11] Karen Jenkins-Keeran, Paul Schreuders, Kimberly Edwards, L. Curry Woods, 2001: The effects of oxygen on the short-term storage of striped bass semen, North American Joumal of Aquaculture 63:238-241.

[12] Moore A.A., 1987: Short-term storage and cryopreservation of walleye semen- Progressive Fish-Culturist 49:40-43.

[13] Nazari R.M., Sohrabnejad M., Ghomi M.R., 2009: The effect of maternal size on larval characteristics of Persan sturgeon *Acipenser persicus*. Aquactic Research 40:1083–1088

[14] Persov G.M., 1941: An account of sturgeon culture work with reference to the use of the method of pituitary injections. In N.L. Gerbil'sky, ed. The method of pituitary injections and its role in reproduction of fish resources. Leningrad, Izdtel'stvo LGU. pp. 42–45.

[15] Rurangwa E., Kime D.E., Ollevier F., Nash J.P., 2004: The measurement of sperm motility and factors affecting sperm quality in cultured fish. Aquaculture, 234, 1-4, 1-28.