SPERM MOTILITY MULTIPLE ACTIVATIONS: PHYSIOLOGICAL BACKGROUND AND PRACTICAL USE IN AQUACULTURE

Boryshpolets S.[a*], Dzyuba B.[a,b], Rodina M.[a]and Linhart O.[a]

a - University of South Bohemia, Faculty of Fisheries and Protection of Waters, CENAKVA, Zatisi 728/II, 389 25 Vodnany, Czech Republic

b - Institute for Problems of Cryobology & Cryomedicin of National Academy of Sciences of the Ukraine, Kharkov, UkraiIntroduction

* - fax: +420387774634, e-mail: boryss00@vurh.jcu.cz

Introduction:

In freshwater fish spawning, sperm motility activation is related to low osmolality of the environment, its duration lasts for short periods and the lost motility is associated with disappearance of fertilizing ability [5]. During artificial reproduction there is the possibility to increase the duration of sperm motility [4] and even restore the motility of spermatozoa being immotile [6]. In this report we summarized our results on studying the physiological backgrounds for sperm motility multiple activation in fish spermatozoa and appearance of spontaneous activations during cryopreservation. Further, we discuss the possible advantage of this phenomenon when necessary to improve the results of artificial reproduction in fish.

Methods:

Fish and sperm sampling- Mature males of Eurasian perch and Common carp were obtained after fish farm pond harvesting and kept in laboratory aquatic systems. Sperm samples were obtained during natural spawning period in case of perch while in carp it was collected after treatment with carp pituitary extract.

Sperm motility parameters- Sperm velocity and motility (percentage of motile spermatozoa) were estimated using an analysis of video records obtained by applying CCD video camera mounted on a dark-field microscope and illuminated with a stroboscopic lamp. Motility was initiated either by sperm dilution in hypotonic media or by freeze-thawing.

Models of sperm multiple activation (1) motility of carp sperm was initiated in hypotonic activating media (100-150 mOsm), after motility stop KCl was added to increase the osmolality up to 300 mOsm and after 20 min the second motility activation by osmolality decrease was initiated; (2) motility of perch spermatozoa was initiated 3 times using stepwise reduction of osmolality of activating solution; (3) motility arisen from freeze-thawing process was observed in carp and perch without transferring them into activating media.

Sperm cryopreservation- Sperm samples were cryopreserved using specific cryoprotective media and freezing methods described previously.

Sperm ATP content measurements- ATP content was evaluated by bioluminescence using a Bioluminescence Assay Kit and multifunctional microplate reader.

Results and discussion:

The different modes of multiple sperm motility activation were investigated. (1) After motility stop following the transferring of carp sperm into isotonic condition the ATP level and cellular volume can be recovered together with ability for the second motility activation in hypotonic condition. During the second activation the gradual increase of motility percentage was observed, while in firstly activated spermatozoa the maximum motility observed right after the start of movement. The rate of motility percentage in secondly activated spermatozoa is associated with individual properties of sperm samples (fig 1.) [1]. These secondly activated spermatozoa have the fertilizing ability [6] and survived cryopreservation demonstrating up to 20% of motility after thawing. The respiration rate during resting period was significantly higher than in immotile sperm and not different from respiration rate during motility or uncoupling condition [1]. That is why we suppose that at least part of ATP produced during reactivation were supported by oxidative phosphorylation.

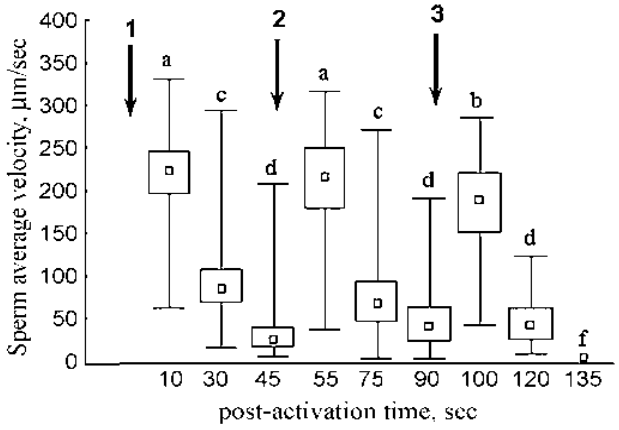

Fig. 3.Perch sperm average velocity during motility activation) in the model of multiple activation.
Arrows – first, second and firth motility activation correspondingly.

Second mode (2) consists of stepwise decreasing the osmolality of the activating medium. During this kind of multiple activation the total duration of motility is around 3 times longer if compared with activation by sharp osmolality decrease. This phenomenon is based on prolonged and slow ATP consumption during several "waves" of motility arisen due to stepwise environment osmolality decrease (fig.2) [2]. Third mode (3) of multiple activation was observed in carp and perch, when procedure of freeze - thawing itself leads to spontaneous sperm motility activation and endogenous ATP level decrease. Afterwards the sperm could be reinitiated in hypotonic conditions. During this useless activation spermatozoa lost the main part of ATP but still able to be secondly activated and preserve ability for fertilization [3].

Conclusion:

The phenomenon of multiple sperm activation could be the base for the elaboration of the most optimal sperm use because the prolongation of total sperm motility duration potentially and more likely makes the fertilization successful. Spontaneous sperm motility activation during freeze-thawing should be taken into account during cryopreserved sperm use. Finally, conjunction the ability of sperm for multiple activation and its cryopreservation could be at the base of multiple sperm use for fertilization if there is a deficit of valuable individuals' sperm.

Acknowledgements:

Special thanks are expressed to the projects: ME10015, QH82119 and LC06073, CZ.1.05/2.1.00/01.0024, GACR 502/11/0090, GAJU 046/2010/Z.

References:

[1]BORYSHPOLETS S., DZYUBA B., DROKIN S. 2009a. Pre-spawning water temperature affects sperm respiration and reactivation parameters in male carps. Fish Physiol. Biochem. 5:661–668.

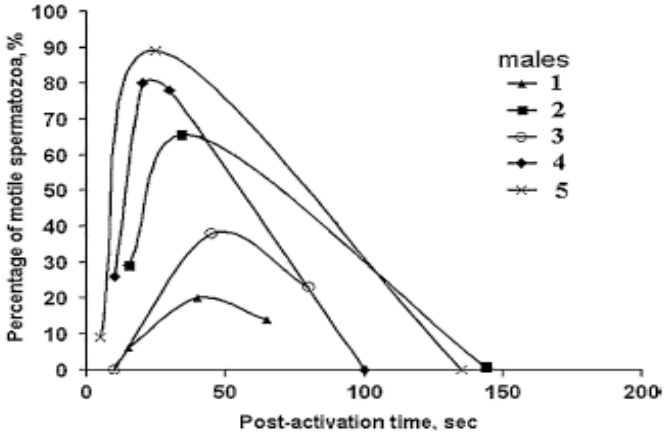

Fig. 1. Graph showing percentage of motile spermatozoa (%) at the second activation obtained for five different males

[2]BORYSHPOLETS S., DZYUBA B., STEJSKAL V., LINHART O. 2009b. Dynamics of ATP and movement in Eurasian perch (*Perca fluviatilis* L.) sperm in conditions of decreasing osmolality. Theriogenology, 72 : 851–859.

[3]BORYSHPOLETS S., DZYUBA B., RODINA M., LI P., HULAK M., GELA D., LINHART O. 2009c. Freeze-thawing as the factor of spontaneous activation of spermatozoa motility in common carp (*Cyprinus carpio* L.) Cryobiology 59: 291–296

[4]COSSON J. 2004. The ionic and osmotic factors controlling motility of fish spermatozoa. Aquacult. Int.,12:69–85.

[5]GINZBURG A.S. 1972. Fertilization in fishes and problem of polyspermia. US Dep. Comm. Nat. Tech. Inf. Ser. Springfield. p366.

[6]LINHART O., ALAVI S.M.H., RODINA M., GELA D., COSSON J. 2008. Comparison of sperm velocity, motility and fertilizing ability between firstly and secondly activated spermatozoa of common carp (*Cyprinus carpio*). J. Appl. Ichthyol.,24:386–92.