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Швидкість запліднення та частота аномалій личинок морського їжака *Paracentrotus lividus* після використання кріоконсервованої сперми

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Fertilization Rate and Larvae Abnormalities Rate for *Paracentrotus Lividus* Sea Urchin When Using Cryopreserved Sperm

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The keeping of laboratory animals, which are model objects of biotechnological research, is associated with certain problems, such as seasonality of access to mature animals, their genetic heterogeneity, which may affect the results of scientific research. Cryopreservation of gametes, in particular sperm, allows to preserve genetic material for a long time and maintain animal breeding in the laboratory throughout the year.

The aim of this study was to evaluate the fertilization rate and the larvae abnormalities rate of the sea urchin *Paracentrotus lividus* after the use of cryopreserved sperm.

Sperm of *Paracentrotus lividus* were obtained using the "dry" method, avoiding contact with water, into plastic tubes. Sperm motility and concentration were determined immediately. Aliquots of semen (200 µl) were mixed with 30% solution of DMSO in filtered sea water in a ratio of 1:1 with a final DMSO concentration of 15%, equilibrated for 5 min at room temperature (20°C) and filled into 0.25 ml straws. The straws were placed 5 cm above the liquid nitrogen and kept for 8 minutes, then plunged into liquid nitrogen. Thawing of straws was performed in a water bath at 35°C for 6 s. Spermatozoa were washed from the cryoprotective medium by gradually adding filtered seawater to restore motility of more than 65%. Fresh oocytes were mixed with sperm at the rate of 10 sperm per 1 egg. One hour later, the fertilization rate was determined, then the cells were cultured at 18°C. On the second day of cultivation, the abnormal larvae rate was calculated. The control group was a group of sea urchin oocytes fertilized with fresh sperm.

The use of cryopreserved sperm significantly ($p \leq 0.05$) reduced the frequency of oocyte fertilization, compared with the fertilizing ability of fresh sperm. Thus, the fertilization rate using cryopreserved sperm was almost 40% lower than with fresh sperm. Analysis of morphological characteristics of sea urchin larvae on the second day of cultivation did not reveal a significant difference in the larvae abnormalities rate.

To conclude, after cryopreservation of sea urchin sperm, there is a decrease in their fertilizing ability, which is manifested in a decrease in fertilization rate. However, the larvae abnormalities rate after using cryopreserved sperm for oocyte fertilization was at the same level as the fresh sperm.

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Оцінка взаємодії фізико-хімічних факторів при кріоконсервуванні личинок *Paracentrotus lividus*

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Evaluation of Physico-Chemical Factor Interactions in the Cryopreservation of *Paracentrotus lividus* Pluteus Larvae

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The sea urchin *Paracentrotus lividus* is a species of high economic, ecological and scientifi c value [Rey-Méndez *et al.*, 2015]. Currently, there is already a successful protocol for the cryopreservation of embryos [Paredes and Bellas, 2015] but not for pluteus larvae. Obtaining a protocol for the cryopreservation of pluteus larvae will guarantee improvements in their culture or their applications in bioassays [Bellas and Paredes, 2011]. In the present work, we aim to achieve a successful protocol for the cryopreservation of *P. lividus* pluteus larvae by modulating two stress factors (salinity and temperature) whose ranges have been previously determined by bioassays using four pre-freezing pre-treatments (18ºC – 29.5‰; 18ºC – 35‰ and 20ºC – 29.5‰; 20ºC – 35‰). In addition, toxicity tests were performed with different cryoprotectants: methanol (METH), ethylene glycol (EG), propylene glycol (PG), dimethyl sulfoxide (Me_2SO) and glycerol (GLY), in a range of 0.5–3M, best results pointed out to METH and Me_2SO as those suitable for cryopreservation. Finally, a cryopreservation experiment was performed with both cryoprotectants supplemented with 0.04M trehalose on 4-arm pluteus larvae (48h-old) developed in these pre-treatment conditions, followed by a simpler and shorter protocol than the one used for embryos [Paredes and Bellas, 2015] with a cooling rate of 1ºC/min to –35ºC, achieving for the first time the successful cryopreservation of *P. lividus* larvae. Contrary to what we expected, no improvements were observed by incubation on those pre-treatment conditions of low salinity or temperature, on the contrary delays in larval development were observed. Me_2SO was the cryoprotectant that showed effective cryoprotection of the larvae similarly to data from Paredes and Bellas (2009), when dimethyl sulfoxide despite not being the less toxic compound still resulted in the best cryopreservation outcome. Damage of the larval structure frost-thaw was studied with light and fluorescence microscopy.