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## Труднощі кріоконсервування та ефективність кріопротекторів при заморожуванні ембріонів *Cyprinodon* variegatus

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## Cryopreservation Challenges and Cryoprotectant Efficacy in *Cyprinodon variegatus* Embryos

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*Cyprinodon variegatus* is a species of actinopterygian fish native to the eastern coasts of North and Central America, known for its high thermal tolerance. Despite its significance, there is a lack of research on the cryopreservation of this species, and successful cryopreservation of fish eggs or embryos remains a challenge.

Three studies were undertaken using three different cryoprotective agents (CPAs): ethylene glycol (EG), dimethyl sulfoxide (DMSO), and methanol (MET), all of which are known for their membrane-permeable properties. The first study examined the toxicity of these CPAs at varying concentrations (1 mol/L, 1.5 mol/L, and 2 mol/L) at a control temperature of 25°C over a 96-hour exposure period. The second study investigated sensitivity to low temperatures (3°C) in control groups with a salinity of 28‰, also over a 24-hour exposure period. Finally, the third study explored sensitivity to both low temperatures (3°C) and CPAs at different concentrations (0.5 mol/L, 1 mol/L, and 1.5 mol/L) over 96 hours.

The results of the first experiment revealed a significant disparity in mortality rates between the control group and those exposed to CPAs, indicating that CPAs adversely impact embryo survival irrespective of temperature, particularly with MET displaying toxicity to embryos. The second experiment corroborated findings from previous studies by Bennett and Beitinger (1997), affirming that *Cyprinodon variegatus* demonstrates a wide tolerance range for temperatures.

In the third experiment, it was observed that mortality rates were significantly higher ($P < 0.05$) in the control group at 25°C compared to those at 3°C. Furthermore, mortality was significantly higher ($P < 0.05$) in the control group at 25°C than in the group treated with DMSO at a concentration of 1.5 mol/L. Complete mortality was observed in plates treated with EG and MET, regardless of concentration. Embryos incubated at 25°C from the outset hatched significantly earlier ($P < 0.05$) than those incubated at 3°C, indicating temperature's influence on metabolic activity and life cycle progression. Additionally, hatching rates were higher in the group maintained at 25°C from the start compared to the control group initiated at 3°C, underscoring the detrimental impact of low temperatures on egg hatching. However, plates treated with DMSO at concentrations of 0.5 mol/L and 1 mol/L achieved hatching percentages comparable to or even better than the control group at 25°C, and consistently higher than the control group at 3°C, suggesting DMSO's effectiveness as a cryoprotectant.

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## Міграційна відповідь астроцитів на дію кондиціонованого середовища, отриманого з мезенхімальних стовбурових клітин кісткового мозку щурів

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## Migration Response of Astrocytes to the Effect of Conditioned Medium From Rat Bone Marrow Mesenchymal Stem Cells

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Mesenchymal stem cells are considered as a source of bioactive substances capable of inducing survival and regeneration of various cell types. The aim of this study was to characterize the conditioned medium (CM) from rat bone marrow mesenchymal stem cells (BMMSC) obtained at different conditioning times and to assess its impact on the migratory response of astrocytes in a scratch model of mechanically injured primary cultures of nerve cells.

Conditioned media were prepared by conditioning rat BMMSC for 24 hours (CM24), 48 hours (CM48), and 72 hours (CM72) in serum-free media and subsequently frozen at –80°C until use. After one week the samples were thawed and proteomic profiles of individual CM were analyzed using mass spectrometry. The concentrations of four selected neurotrophins (BDNF, NGF, GDNF, and VEGF) were determined by ELISA. In the *in vitro* part of the experiment, primary cell cultures isolated from the spinal cords of 4-day-old Wistar rats were used. Cultures were mechanically injured by scratching on day 7 and then cultured in conditioned media for 3 days. The effect of different CMs was evaluated by monitoring the migratory response of astrocytes – measuring the width of the cell-free linear area (created by scratching) from one edge to the other in groups influenced by CM compared to the control (without CM).

Proteomic analysis revealed variability in the number and representation of identified specific proteins depending on the conditioning time, with higher protein representation observed in CM72. ELISA showed a gradual increase in the concentration of two neurotrophins (NGF, VEGF) with maximum concentrations in CM72. In the *in vitro* conditions, a differential effect of various conditioned media on the migratory response of astrocytes was observed, with these effects being most pronounced in the group influenced by CM72.

Our results indicate that prolonged conditioning leads to increased release of detectable proteins and elevated concentrations of certain neurotrophins (NGF, VEGF) in individual CM. The findings from the *in vitro* part of the experiment suggest that media obtained after long-term conditioning may have a more significant impact on repair processes in nerve cell cultures.

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