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Cryopreservation of equine and canine monocyte derived dendritic cells

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Cell-based immunotherapies offer significant potential in veterinary medicine, yet their widespread clinical use is hindered by the limited shelf life of cell suspensions. Cryopreservation of autologous donor cells presents a practical alternative to repeated blood collection, reducing logistical challenges and improving animal welfare. However, this approach may compromise post-thaw cell viability and recovery. In this study, we evaluated cryopreservation protocols for equine and canine monocyte-derived dendritic cells (Mo-DCs).

For equine Mo-DCs, a slow-freezing protocol (1 K/min) using a controlled-rate freezer and a cryoprotectant consisting of 10% (v/v) DMSO and 90% (v/v) autologous serum showed no significant differences in cell recovery or viability across varying cooling rates (~0.5 K/min, ~0.75 K/min, and 2 K/min) or storage temperatures (−80 °C *vs*. liquid nitrogen, −196 °C) for up to 8 weeks. Passive cooling devices yielded comparable results. However, thawing at elevated temperatures (50 °C for 80 s and 65 °C for 100 s) improved recovery compared to the standard 37 °C for 2 min. Reducing DMSO concentration (7.5, 5, and 1% v/v) also yielded promising outcomes. Functional assays confirmed that cryopreserved equine Mo-DCs retained their expected functionality.

Applying the standard protocol to canine Mo-DCs produced highly promising results. Storage at −80 °C demonstrated comparable recovery rates to −196 °C, both immediately post-thaw and after 24h of hypothermic transport/storage (2—8 °C). However, both groups exhibited a significant decline in recovery following hypothermic conditions.

These findings suggest that optimized thawing conditions and alternative cryoprotectant formulations can enhance Mo-DC cryopreservation. Future work will focus on improving logistical conditions by refining hypothermic storage solutions and advancing transport container technology.

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Cryopreservation of fish embryos: insights from *Cyprinodon variegatus*

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Cyprinodon variegatus is a eurythermal species of actinopterygian fish native to the eastern coasts of North and Central America, known for its high tolerance to temperature and dehydration. Although fish embryo cryopreservation has been achieved punctually in zebrafish, the unique physiological characteristics of marine fish embryos, particularly their higher water content — pose additional challenges, including increased susceptibility to intracellular ice crystal formation.

The study aimed to evaluate the effects of dehydration and cryoprotection using dimethyl sulfoxide (DMSO) on the survival of *C. variegatus* embryos, as a preliminary step toward developing a viable cryopreservation protocol. Two experimental approaches were undertaken to determine the tolerance to cell dehydration and DMSO cryoprotection in the survival rate. Dehydration's effect was assessed using a solution of sucrose 2M, comparing the survival rate at different dehydration times, from 30 minutes to 180 minutes. Cells were stepwise returned to the normal volume and survival was evaluated by analysing normal development and hatching rates. On other hand, the effect of dehydration was tested while adding also DMSO in a secondary step (2/3 of the time of immersion in sucrose 2M and 1/3 in a solution of 2M of sucrose 1M DMSO). Cells were stepwise returned to the normal volume and survival was evaluated by analysing normal development and hatching rates at 10 minutes, 3 and 5 days of incubating the eggs at 25.5 °C. Results showed that there was not an observable impact to the survival rates and hatching rates ($P > 0.05$) after dehydrating or dehydration plus DMSO.

The next phase of this research will explore the use of vitrification following dehydration and DMSO loading, in an effort to overcome the limitations observed in traditional cryopreservation methods. Even in the absence of immediate success, this study contributes valuable data for future investigations into the cryopreservation of marine fish eggs.

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