

COMPARATIVE STUDIES OF DIFFERENT CRYOPRESERVATION METHODS FOR CHONDROCYTES AND MYOCYTES

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Proper cell cryopreservation from endangered domestic species is very important as these cells could be used to reintroduce lost genes back into the breeding pool by somatic cell nuclear transfer. In this study we have investigated the effect of different cryopreservation methods and cooling rates on primary bovine chondrocyte/myocyte viability and proliferative activity. Cells were cooled in %10 DMSO freezing media at 1°C/min, 2°C/min and 0,5°C/min rate or vitrified in a solution composed of 40% EG, 18% Ficoll and 0.3 M sucrose. Viability ratios of thawed cells were evaluated by trypan blue staining and post-thawing proliferative activity was measured by MTT assay. Results showed that slow freezing at 1°C/min cooling rate was the most appropriate method for the cryopreservation of myocytes ($p < 0,05$). The viability ratios of cryopreserved chondrocytes by slow freezing at 1°C/min and 2°C/min cooling rate were higher than those cryopreserved by slow freezing at 0,5°C/min or vitrification. Cell viability decreased when 5% DMSO concentration was used at 1°C/min cooling rate. Post-thawing viability ratios of chondrocytes and myocytes were 78% and 74% at 1°C/min; 80% and %51 at 2°C/min; 61% and %51 at 0,5°C/min; 61% and 49% at vitrification. Post-thawing proliferative activity of vitrified chondrocytes and myocytes were lower than slow freezing groups. Results revealed that viability at different freezing rates and cryoprotectant concentrations changed according to the cell type. Cryopreservation protocols should be optimized for specific cell types due to different cryosensitivities.

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