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Abstracts



cells within intact and mechanically stable alginate micro-spheres. Alginate-based semipermeable capsules allow for permanent protection of cells against physical damage caused by the cryopreservation process. In this work, a range of parameters such as alginate cross-linking time, concentration of dimethyl sulfoxide, its loading time and cooling rate were analysed for freezing of 300, 800, 1000 and 1500 µm alginate micro-spheres. The micro-spheres were generated using electro-spraying (300 µm) and air flow (800, 1000 and 1500 µm) encapsulation methods. The structural integrity of cell-free alginate micro-spheres was evaluated upon freeze-thaw cycles using an Axio Imager M1m microscope with Linkam cryostage to identify an optimal combination. Verification of optimal cryopreservation protocol yielding intact capsules has been performed using multipotent stromal cells derived from the common marmoset (Callithrix jacchus). Freezing of alginate-encapsulated cells has been conducted using a controlled-rate freezer Planer Kryo 560-16 and Askion C-Line workbench. Analysis of cell viability after thawing has been performed using Calcein-AM / EthD-1 cell viability assay following image analysis using µVision software. Among the optimal conditions, the following ones yielded the highest cell viability (83%) and recultivation efficiency (70%) 24 h after thawing: cross-linking time 15 min, 10% (v/v) Me2SO, loading time at 4°C of 45 min and a cooling rate of 2.5 K/min. The results of this study demonstrate a high potential for clinical application of mechanically stable alginate capsules for efficient treatment of rare diseases.

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P52 EFFECT OF VITRIFIED-WARMED BOVINE OOCYTES ON THE BLASTOCYST HATCHABILITY

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This study evaluates the effect of vitrification of blastocyst which derived from IVF following vitrified-warmed matured bovine oocytes. During experiment, IVM oocytes or blastocysts were exposed to medium containing 5% dimethylsulfoxide (DMSO) and 5% ethylene glycol (EG) for 30 sec, followed by exposed to medium containing 12% DMSO, 12% EG and 0.25M sucrose for 30 sec, finally exposed to vitrification solution containing 20% DMSO, 20%EG and 0.5M sucrose for 30 sec. Oocytes or blastocysts were loaded on a paper container before plunged into liquid nitrogen. Oocytes were conducted into four groups; A) fresh MII oocytes and blastocysts, B) vitrified IVF-derived blastocysts, C) vitrified MII oocytes only and D) vitrified both MII oocytes and blastocysts. The results revealed that blastocyst production rates of group A, B, C and D were 15.7, 16.5, 9.4 and 9.4%, respectively, which was not significant difference. In case of blastocysts hatchability rate, the blastocysts in group A, B, C and D were 73.3, 56.5, 66.6 and 16.7%, respectively. The blastocysts hatchability rates of blastocyst of group C and D were not difference when compared with group A. However, blastocysts hatched rate in group C (6.3%) was significantly ($p<0.05$) lower than that of Group A (12.1%), but significantly ($p<0.05$) higher than that of group D (1.6%). We estimated that the oocytes survival rate in group C was significantly correlated with cleaved rate whereas the blastocysts hatchability rate in group A showed significant correlation ($P<0.01$) with its survival rate. In conclusion, the blastocyst hatched rate was seriously affected by double vitrification on oocytes and blastocysts.

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P53 GLYCEROL AND POSTHYPERTONIC SHOCK OF ERYTHROCYTES WHEN VARING MEDIUM TEMPERATURE AND OSMOLALITY

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In this research the effect of glycerol on sensitivity of human erythrocytes to the action of posthypertonic shock (PHS) was studied. PHS was initiated by transferring the erythrocytes from dehydration medium (1.5 mol/L NaCl) into rehydration one (0.15 mol/L NaCl) The experiment was carried out in two versions: 1) the erythrocytes pre-treated with glycerol (37°C, 20 min) were subjected to PHS (control); 2) glycerol pre-treated cells were also exposed to PHS, but glycerol was present in the dehydration medium. In the presence of glycerol (15%) the incubation time of erythrocytes at the dehydration and rehydration stages of PHS were 20 and 5 mins' respectively. At 37°C, when the glycerol concentrations (5, 10 and 15%) were varied, an increase of hemolysis level of erythrocytes was shown with using the cryoprotective agent (CPA) in a high concentration (compared to cells without CPA). The presence of glycerol in the dehydration medium led to an increase posthypertonic hemolysis of erythrocytes approximately in 1.5 times at 10 and 15% CPA. At 0°C the addition of glycerol (5, 10 and 15%) resulted in the development of a high level of posthypertonic hemolysis of the control cells and erythrocytes, which were transferred to the dehydration medium containing CPA. The obtained temperature dependencies of posthypertonic hemolysis of cells pre-treated with CPA (15%) show that under low

temperatures (0 - 5°C) the hemolysis values are approximately 3.5 times higher than those for untreated with CPA erythrocytes, and within the temperature range from 5 to 30°C there is a uniform decrease in the hemolysis values from 90 down to 25%.

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P54 CATHEPSIN B-LIKE CYSTEINE PROTEASE APCATHB ADVERSE IMPACTS CELL VIABILITY IN CRYOPRESERVATION

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Cryopreservation-induced cell death is regarded as an important drawback faced by cryobiologists. We isolated a gene, encoding cathepsin B-like cysteine protease from Agapanthus
praecox ssp. orientalis named ApCathB, whose expression pattern had a positive correlation to the occurrence of programmed cell death (PCD) during cryopreservation. The mature ApCathB protein possess in vitro cathepsin B activity and its activity could be inhibited by E-64 and Leupeptin. Overexpression of ApCathB in A. praecox embryogenic callus (OXEC) reduced the survival rate from 45.68% to 18.33% after cryopreservation. Along with the reduced cell viability, the H2O2 and O2·- significantly accumulated in OXEC. The DNA fragment detection showed intensified signal by TUNEL and higher ratio of DNA ladder during OXEC cryopreservation. The cis-elements of TCA-element, ERE, MBS and ARE were responsible for driving the ApCathB expression during cryopreservation treatment. This indicated that the expression of ApCathB was induced by the environment stress of drought and hypoxia and the hormone signals of salicylic acid and ethylene. We analyzed the differentially expression genes (DEGs) of transgenic (OXEC) and non-transgenic (NTEC) embryogenic callus treated with dehydration, dilution and 24 h-recovery to investigate the downstream events effected by ApCathB during cryopreservation. The oxidation reduction process and MAPK cascade were altered in OXEC cryopreservation. Overexpression of ApCathB weakened the antioxidant ability of the cells. In addition, as another partial of stress defense system, the second metabolism and signal transduction pathways were also down-regulated in OXEC. In cryopreservation-induced PCD pathway, ApCathB aggravated the protein proteolytic and inhibiting the anti-apoptosis genes expression. This work verified that ApCathB negatively impact the cell viability through mediate the PCD in plant cryopreservation and provided a novel insight into the mechanism of PCD in cell viability during cryopreservation.

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P55 CRYOPROTECTIVE AGENTS AND LOW TEMPERATURE AFFECT THE ERYTHROCYTE SURFACE MARKER CD44

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Changes in surface markers induced by cryopreservation stresses can be an important sign of cell injuries. This study aims to examine changes in the marker CD44 in erythrocytes under the effect of cryoprotective agents (CPAs) and the impact of cross-linked modification of membrane-cytoskeleton proteins on CD44 over the cell exposure to CPAs, as well as the assessment of consequences of erythrocyte cryopreservation for CD44 alterations. Prolonged exposure of erythrocytes (20 h) with glycerol, Me2SO, sucrose and PEG-1500 led to a reduced CD44 expression and decrease in the CD44+-cell amount in contrast to the short-term exposure, when no changes were found. Extracellular CPAs exhibited a more pronounced effect on erythrocyte CD44 in comparison with the examined endocellular substances. Modification of membrane-cytoskeleton proteins with protein-linking reagent diamide intensified the revealed tendencies. Meanwhile, short-time incubation (1h, 37oC) of frozen -thawed erythrocytes caused a significant decrease in the CD44 expression and in the CD44+-cell amount. These changes were more pronounced in erythrocytes cryopreserved with PEG-1500 than with glycerol. Evidently, time discrepancies in the manifestation of the CD44 changes in erythrocytes exposed to CPAs and cryopreserved ones specify the different processes affecting the cell stability. The changes revealed during cell exposure to CPAs may attest subtle adjustments in protein-protein interactions in erythrocyte membrane-cytoskeleton complex, which are able to increase the cell resistance. Whereas the CD44 alterations in cryopreserved erythrocytes can attest membrane injuries. Since cryopreserved erythrocytes that survived after CPA removal were found to have similar CD44 characteristics as fresh erythrocytes indicating that changes in erythrocytes covered only part of the cells, which were instable and lysed during the washing process. Thus, changes in surface markers of erythrocyte membranes caused by CPAs and freeze-thawing processes can be important for the assessment of structural and functional cell integrity.

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P56 CONVOLUTIONAL NEURAL NETWORK-BASED CELL SEGMENTATION DURING FREEZING IN CRYOPRESERVATION

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