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Cell Cycle and Apoptosis of Bovine Fetal Fibroblast Cells following Different Activation Treatments

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The success of embryo cloning depends on numerous factors; interaction between recipient ooplasm and donor nucleus, nuclear reprogramming, oocyte activation, and donor cell cycle and type. In this study, the cell cycle and apoptosis of bovine fetal fibroblast as a donor cell for embryo cloning were evaluated following different activation treatments.

Bovine fetal fibroblasts (3 cm of crown rump length) were cultured in DMEM medium + 10% FBS, 1% non essential amino acid and 10 μM 2-mercaptorthanol. Fetal fibroblasts (passages 3-8) were harvested using 0.05 % trypsin-EDTA when they reached to confluent, and frozen in DMSO. After thawing, the cells were assigned to 4 experimental groups by different activation treatments (Group 2, un-treated; Group 3, pulsed single by electric with 1.6 KV/cm, 60 μsec in 0.3 M mannitol soln; Group 4, electric + 1.9 mM 6-dimethylaminopurine (DMAP) for 3 h; Group 5, electri + 10 μg/ml cycloheximide (CHX)/5 μg/ml cytochalasin B (CCB), and compared to control fresh cells (Group 1). At 0, 1, 2 and 3 h after treatment, the cells were fixed in 70% ethanol and stored at -20℃ until used. Cells were analyzed the cell cycle by flow-cytometry, and stained with YO-PRO-1/propidium iodide (PI) (group 1, 2) for detection of apoptosis under a fluorescence microscope. Differences between treatments were analyzed by ANOVA program ($P<0.05$). The results of cell cycle analysis appeared that frozen-thawed cells (group 2) was G0/G1 phase rate higher than fresh cells (Group 1), (92.20% vs. 84.25%). Group 4, the rate of G2/M phase higher than that of group2 and group 4 at 0h after treatment The incidence of cell apoptosis in frozen-thawed cells (Group 2) was significantly ($P<0.05$) higher than those in fresh cells (Group 1). These results suggest that, in addition to the negative effects of cell frozen causing by detrimental embryonic development of cloning embryos, activation reagents influence donor cell cycle.

Key words) ***apoptosis, cell cycle, flow-cytometry, YO-PRO-1/propidium iodide (PI)***