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Developmental Efficiency of Bovine Embryos Cloned with Fetal Fibroblast Arrested at G0/G1 Phase

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The study evaluated the effect of donor cell treatments for G0/G1 synchronization and the donor cell type on development and incidence of apoptosis in cloned cattle embryos. Primary cultures were established from a female fetus on day 50 of gestation and adult ear skin biopsies. Cells were randomly allocated into 3 experimental treatment groups after 6~8 passages. Group 1 (Confluent), cells were cultured in DMEM supplemented with 10% FBS until 90% confluent. Group 2 (Serum-starvation), cells were cultured in DMEM supplemented with 0.5% FBS for 5 days. Group 3 (Roscovitine), cells were cultured in DMEM supplemented with 10% FBS and 30 μM Roscovitine for 12 h. Cell cycle and apoptosis were analyzed using flow cytometry after labelling with DAPI and YO-PRO-1. At 19 h post-maturation (hpm), enucleated oocytes were reconstructed with donor cells and fused by a single DC pulse (1.6 kV/cm, 60 μsec). After activation with ionomycin (5 μM, 5 min) and cycloheximide (10 μg/ml, 5 h), the eggs were cultured in CR1aa for 3 days and additionally cultured in CR1aa + 30 mg/ml BSA for 5 days at 39℃ in a humidified atmosphere of 5% CO_2 in air. There were no significantly differences .in the incidence of cells arrested at G0/G1 for fetal fibroblasts cultured in the three treatment groups (87%, 83% and 80%; confluent, serum starvation and Roscovitine, respectively). More cells appeared as apoptotic in Group 2 compared to the cells in Group 1 and 3 (12% vs. 6 and 6%, respectively) ($P<0.05$). Blastocyst development of cloned embryos was significantly ($P<0.05$) higher when fetal fibroblasts from Group 1 were used, compared to Groups 2 and 3 (35.1%, vs. 31 and 29.7%, respectively). Similar results were observed in the use ear skin fibroblasts as nuclear transfer donor cel s (32.7%, vs. 24 and 24%, respectively). These results suggest that fetal fibroblasts can be effectively synchronized at G0/G1 by three different treatments, including growth to confluence, serum-starvation and Roscovitine treatment. However, based on blastocyst development and levels of apoptosis, the use of confluent fetal fibroblasts as donor cells is more effective than using cells synchronized by serum-starvation or Roscovitine treatment in the production of cloned bovine embryos. [Supported by High Technology Development Project for Agriculture and Forestry Korea, MAF-SGRP, 30012-05-3-SB010 and Cho-A Pharm.Co.LTD].

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