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Cell Cycle Analysis of Bovine Cultured Somatic Cells by Flow Cytometry

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The cell cycle phase in which donor nuclei exist prior to nuclear transfer is an important factor governing developmental rates of reconstituted embryos. It was suggested that quiescent G0 and cycling G1 cells could support normal development of reconstituted embryos. In a quest of optimized donor nuclei treatment prior to nuclear transfer, this study was undertaken to examine the cell cycle characteristics of bovine fetal and adult somatic cells when cultured under a variety of culture treatments and the cell cycle change with the lapse of time after trypsinization. This was archived by measuring the DNA content of cells using flow cytometry. Cultured fetal fibroblast cells, adult skin and muscle cells, and cumulus cells were divided by 3 culture treatments; 1) grown to 60-70% confluency (cycling), 2) serum starved culture, 3) culture to confluency. Trypsinized cells were fixed by 70% ethanol and stained with propidium iodide. For one experiment, trypsinized cells were resuspended in DMEM+10% FBS and incubated for 1.5, 3 and 6 h with occasional shaking before ethanol fixation. Cell cycle phases were determined by flow cytometry enabling calculation of percentages of G0+G1, S and G2+M. The majority of cells were in G0+G1 stage regardless of origin of cells. Cultures that were serum starved or cultured to confluency contained significantly ($P<0.05$) higher percentages of cells in G0+G1 (89.5-95.4%). For every cell lines and culture treatments, percentages of cells in existing in G0+G1 increased with decreasing of the cell size from large to small. In the serum starved and confluency groups, about 98% of small cells were in G0+G1. Serum starved culture contained higher percentages of small-sized cells (38.5-66.9%) than cycling and confluent cultures regardless of cell lines ($P<0.05$). After trypsinization of fetal fibroblast and adult skin cells that were serum starved and cultured to confluency, the percentages of cells in G0+G1 significantly increased by incubation for 1.5 (95.7-99.5%) and 3.0 h (95.9-98.6%). The results suggest that the efficient synchronization of bovine somatic cells in G0+G1 for nuclear transfer can be established by incubation for a limited time period after trypsinization of serum starved or confluent cells.

(Key words) **cell cycle analysis, flow cytometry, culture treatment, cell size, bovine somatic cells.**