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Establishment of an Efficient System for the Production of Transgenic Somatic Cell Nuclear Transfer Embryos

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The present study was conducted for the production of transgenic cloned cows by somatic cell nuclear transfer (SCNT) that secrete human prourokinase into milk. To establish an efficient production system for bovine transgenic SCNT embryos, the effect was examined of various conditions of donor cells including cell type, size, and passage number on the developmental competence of transgenic SCNT embryos. An expression plasmid for human prourokinase (pbeta-ProU) was constructed by inserting a bovine beta-casein promoter, a green fluorescent protein (GFP) marker gene, and a human prourokinase target gene into a pcDNA3 plasmid. Three types of bovine somatic cells including two adult cells (cumulus cells and ear fibroblasts) and fetal fibroblasts were prepared and transfected using a lipid-meidated method. In Experiment 1, developmental competence and rates of GFP expression in bovine transgenic SCNT embryos reconstructed with cumulus cells were significantly higher than those from fetal and ear fibroblasts. In Experiment 2, the effect of cellular senescence in early (2 to 4) and late (8 to 12) passages was investigated. No significant differences in the development of transgenic SCNT embryos were observed. In Experient 3, different sizes of GFP-expressing transfected cumulus cells [large (>30 ㎛) or small cell (<30 ㎛)] were used for SCNT. A significant improvement in embryo development and GFP expression was observed when small cumulus cells were used for SCNT. Taken together, these results demonstrate that (1) adult somatic cells could serve as donor cells in transgenic SCNT embryo production and cumulus cells with small size at early passage were the optimal cell type, and (2) transgenic SCNT embryos derived from adult somatic cells have embryonic development potential.

Key words) ***nuclear transfer, transgenic, bovine, human prourokinase***