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Production of Cloned Bovine Embryos Carrying with Human Thrombopoietin Gene

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Human thrombopoietin (hTPO) is a *cytokine* that plays a central role in megakaryopoiesis by influencing on the development and ma,turation of megakaryocyte and platelet production. To induce hTPO production in the mammary gland, expression vector was constructed by combining the promoter of bovine beta-casein gene, cDNA of hTPO and neomycine resistance gene for transfection into fibroblasts. Bovine fibroblast cells derived from female ear skin were transfercted with the expression vector using Lipofectamine (Life Technology, NY). Transected cells resistant to G418 treatment (600 ug/ml) were recovered and colony formation was initiated at 13 days. The colonies with about 1 cm diameter were picked and analysed by PCR. Single transfected cells were individually transferred to enucleated oocytes. After electrofusion, the reconstructed embryos were exposed to calcium ionophore (5uM) for 5 min followed by treatment with 6-DMAP (2.5 mM) for 4h. The nuclear transfer embryos were cultured in CR1aa medium at 38.5C, 5% CO_2 for 7 days. Twenty three of 29 (79.3%) colonies were proved to be hTPO transfectants by PCR. The colonies were further passaged and used to produce transgenic embryos using nuclear transfer. Cleavage and developmental rates of reconstructed embryos to the blastocyst stage were 65.1% and 39.4%, respectively. Of 22 blastocysts that developed from reconstructed embryos with the transfected cell, 20 embryos (90.9%) were positive for hTPO by using PCR analysis. The results suggest that somatic cell nuclear transfer is efficient for production of transgenic embryos.

(Key words) hTPO, transfection, nuclear transfer, PCR