

P-67

Production of transgenic cloned pigs harboring human granulocyte-macrophage colony stimulating factor(hGM-CSF) gene by nuclear transfer

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The mammary gland of transgenic livestock can be used as a bioreactor for producing complex therapeutic proteins. Transgenic female pigs harboring goat β -casein promoter/human granulocyte-macrophage colony stimulating factor(GM-CSF) gene were produced by nuclear transfer. GM-CSF stimulates proliferation and maturation of myeloid progenitor cells giving rise to neutrophilic, eosinophilic granulocytes and monocytes.

In the present study, the developmental potential of porcine NT embryos with cloned fetal fibroblast was evaluated. The hGM-CSF gene was transfected into mouse HC11 cell line for the transient expression and confirmed by RT-PCR and Western blot. The cloned fetal fibroblasts were transfected with hGM-CSF gene and selected with G418 for 2 weeks. Presence of the hGM-CSF transgene was confirmed by PCR, Southern blotting, and fluorescent in situ hybridization analyses. Two hGM-CSF clonal cell lines were used as donor cells for NT. A total of 1,243 NT embryos were transferred into 6 surrogate mothers. Five recipients were confirmed to be pregnant at Day 35 by ultrasonography. Of these, two recipients delivered seven healthy female piglets. These results demonstrate that cloned porcine fetal fibroblasts transfected with hGM-CSF gene can direct full-term development following NT.

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