

O-1 Expression of Foreign Genes in Porcine Embryos Following Nuclear Transfer using Porcine Fetal Fibroblasts Transfected by Retrovirus Vector

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In the present study, we demonstrated successful expression of enhanced fluorescent protein (EGFP), LacZ and neomycin resistant (Neo^R) genes in porcine embryos following nuclear transfer with porcine fetal fibroblasts, which were transfected with EGFP, LacZ and Neo^R genes by retrovirus-mediated infection. Then, these transfected porcine fetal fibroblasts were selected by G418 of 800 µg/ml, and expression of EGFP and LacZ genes were found in all selected fibroblasts. Matured porcine oocytes were enucleated by aspirating the first polar body and adjacent cytoplasm (approximately 30% of ooplasm) using a beveled pipette (30 mm in diameter) in NCSU 23-HEPES containing 0.1% BSA and 7.5 µg/ml cytochalasin B. Nuclear transfer of infected porcine fetal fibroblasts into the enucleated oocytes was accomplished by membrane to membrane method. Reconstructed porcine eggs were cultured in 50 µl of NCSU 23 containing 0.5% BSA and cultured for 4 days then transferred to 50 µl of NCSU 23 containing 10% fetal bovine serum and cultured for 3 days. All eggs were cultured at 39°C in an atmosphere of 5% CO₂ in air for 7 days. Reconstructed porcine embryos developing in vitro were examined to evaluate the expression of EGFP under epifluorescence microscopy using a standard FITC filter set and LacZ by X-gal staining. Expression of both EGFP and LacZ genes was detected in morula and blastocyst stage embryos. Out of porcine eggs (125: EGFP and 130: LacZ) reconstructed by nuclear transfer, 80 (EGFP: 64%) and 68 (LacZ: 52.3%) embryos were cleaved, and among cleaved 19 (EGFP: 23.8%) and 16 (LacZ: 23.5%) embryos developed to the morula and blastocyst stages. Of these morulae and blastocysts, 19 (100%) embryos emitted the green fluorescence and 26 (100%) embryos were stained by X-gal. These results suggest that porcine oocytes reconstructed by nuclear transfer from infected porcine fetal fibroblasts can successfully develop to the blastocyst stage. This novel approach might be applicable for the production of transgenic pigs.

O-2 Successful Nuclear Transfer of Cultured Somatic Cells Obtained from HanWoo (Korean Native Cattle)

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We investigated the possibility of producing calves from transferable bovine embryos obtained