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 neyomycin 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℃ in an atmosphere of 5% CO_2 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

by nuclear transfer using somatic cell (bovine fetal fibroblasts)-derived cell lines in HanWoo. As donor nuclei, bovine fetal fibroblast cells, from the fetus approximately 60 to 70 day old, were used. The cell lines have been maintained in culture for at least 6 passages. These fibroblast cells were confirmed normal by karyotyping. Cells were induced into quiescence by serum deprivation for 3-5 days before nuclear transfer. In vitro matured oocytes were enucleated after 18~20 h of maturation, and enucleation was confirmed under UV light after 10 min of incubation with 5 mg/ml Hoechst 33342. The reconstituted embryos were placed into the fusion chamber (3.2 mm gap; BTX 453, USA) in a solution containing 0.26 M mannitol and aligned manually. Two DC pulses (1 s apart) of 1.8 kV/cm for 15 μs were used to fuse the cells. After the electrical pulse, embryos were exposed to 5 μM ionomycin for 4 min, washed with Hepes-buffered TALP medium for 5 min, and cultured in 1.9 mM 6-dimethylaminopurine (6-DMAP) for 3-4 h. Activated embryos were cultured in modified CR1aa (or TALP). On Day 5, embryos were transferred to fresh CR1aa (or TALP) + 10% fetal bovine serum (FBS). On Day 7, embryo development was recorded. Embryos that developed to the blastocyst stages were transferred into recipient cows on Day 6 to 7 of estrous cycle (estrus=0).

The reconstituted embryos were successfully fused (65.4%; 121/185), cleaved (77.7%; 94/121), and developed to blastocysts (23.4%; 22/94). Eleven blastocysts were transferred into 5 recipients. Two recipients were pregnant, confirmed with ultrasonography at Day 50 to 60, and both of them are ongoing their pregnancy. These results show that the cell derived from HanWoo somatic cell lines can be reprogrammed by nuclear transfer and develop subsequently in vivo as well as in vitro.

O-3 The Isolation of IL-1β Induced Genes in Rat Ovary and the Construction of the cDNA Library

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The process of ovulation comprises oocytes maturation, cumulus expansion and follicle rupture triggered by a surge of LH released from the pituitary gland. By the way we have not be able to understand the whole ovulatory process until now. So it is presumed that cytokines especially IL-1β may play an intermediary role in the ovulatory process. Although the relevance of IL-1β to ovulatory process remains uncertain, several lines of evidences support such possibility. The aim of this study is to confirm that IL-1β is an established mediator of ovulation. To do this we have utilized the technique 'Suppression Subtraction Hybridization' which has been developed for the generation of subtracted cDNA libraries. This techniques combines normalization and subtraction in single procedures and used selectively amplify target cDNA fragments (differentially expressed) and simultaneously suppressed non target cDNA amplification. So we performed this procedure and determined the efficiency of the subtraction process. After that the cDNA products were inserted into a plasmid based vector system. Individual colonies were isolated and placed into the