P-1 In Vitro Development and Remodeling of Porcine Embryos Following Nuclear Transfer using Porcine and Mouse Fibroblasts

S. J. Uhm[1], H. M. Chung[2], H. T. Lee[1] and K. S. Chung[1]

[1]Animal Resource Research Center, Kon Kuk University [2]IMC of CHA General Hospital, Pochon CHA University, Seoul, Korea

The objective of this study was that the transfer of nuclei from on cell to another provided a powerful tool for studying the interactions between the cytoplasm of one cell and the nucleus of another. Thus, we determined the developmental capability of porcine fibroblast (PF; from a 35-day-old male fetus) and mouse fibroblast (MF; from a 3 week-old male) after transferring into enucleated porcine oocytes. Nuclear transfer of PF and MF into the enucleated porcine oocytes was accomplished by membrane to membrane method. Reconstructed porcine eggs were cultured in 50 μl of NCSU 23 containing 0.5% BSA for 4 days and then cultured in 50 μl of NCSU 23 containing 10% fetal bovine serum for 3 days. Nuclei of porcine eggs nuclear transferred by PF and MF were developed to the mitotic division and 2 cell stages at 24 hr. Porcine 2-cell embryo reconstructed by MF showed the normal 20XY by chromosome analysis. However, the development capacity of porcine embryos reconstucted by donor cells from two different species showed significantly difference ($p<0.05$) between PFs and MFs. The developmental rate to the morula to hatching blastocyst stages (37.4% of cleaved embryos) of reconstructed eggs using PFs was significantly higher than that of reconstructed eggs using MFs (7.5% of cleaved embryos). Therefore, our finding suggests that mechanisms regulating early embryonic development may be conserved among mammalian species. In addition, porcine oocyte cytoplasm can successfully reprogram the somatic nuclei of different species and develop up to the blastocyst stage in our culture system.

P-2 Integration of EPO Gene in Bovine Embryos Following Nuclear Transfer using Bovine Fetal Fibroblasts Transfected by Retrovirus Vector

C. S. Ryu[1], S. J. Uhm[1], K. S. Kil[1], J. H. Um[1], K. H. Chung[1], H. C. Lee[1], T. Kim[2], H. T. Lee[1] and K. S. Chung[1]

[1]Animal Resource Research Center, Kon Kuk University, Seoul
[2]Department of Physiology, Catholic University of Taegu-Hyosung, Taegu, Korea

The present study demonstrated the successful integration of erythropoietin (EPO) and neyomycin resistant (Neo^R) genes in bovine embryos following nuclear transfer using transfected bovine fetal fibroblasts with these genes by retrovirus-mediated infection. First, transfected bovine fetal fibroblasts were selected by G418 of 800 μg/ml. Then matured bovine 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 PBS containing 0.1% BSA and 7.5 μg/ml cytochalasin B. Nuclear transfer of transfected bovine fetal fibroblasts into the enucleated oocytes was accomplished by membrane to membrane method. Fused eggs were activated by treatment of Ca inophore and 6-DMAP. Reconstructed bovine eggs were cocultured with bovine oviduct epithelial cells in 500 μl of CR1aa containing 10% FBS for 8 days. All eggs were cultured at 39℃ in an atmosphere of 5% CO_2 in air. We examined the reconstructed bovine embryos developed in vitro culture system to determine the inetegration of EPO by PCR. Out of 203 bovine eggs reconstructed by nuclear transfer 167 (58.2%) embryos were cleaved, and among cleaved embryos, 19 (11.2%) embryos developed to the blastocyst stage. Of these blastocysts, 100% integration of EPO gene in 19 embryos was determined by PCR. Therefore, this result indicates that bovine oocytes reconstructed by nuclear transfer from transfected bovine fetal fibroblasts can successfully develop to the blastocyst stage. Futhermore, this novel procedures presumably be a attractive method to produce efficiently the transgenic cattles in the future.

P-3 Production of Transgenic Bovine Embryo by Microinjection of Retroviral Vector into Perivitelline Space

J. Y. Yoon[1], S. J. Uhm[1], K. H. Chung[1], S. H. Kim[1], B. S. Kwon[1], Y. T. Heo[1], T. Kim[2], H. T. Lee[1] and K. S. Chung[1]

[1]Animal Resource Research Center, Kon Kuk University, Seoul
[2]Department of Physiology, Catholic University of Taegu-Hyosung, Taegu, Korea

A critical requirement for integration of retroviruses, other than HIV and possibly related lentiviruses, is the breakdown of the nuclear envelope during mitosis. Nuclear envelope is reforming immediately after cell division, thereby permitting the translocation of the retroviral preintegration complex into the nucleus and enabling integration to proceed. In the oocyte, during metaphase II (M II) of the second meiosis, the nuclear envelope is also absent and the oocyte remains in M II arrest for a much longer period of time compared with M-phase in a somatic cell. Thus, this study was investigated the possibility for the production of transgenic bovine embryos by microinjection of retroviral vector (LNC-EPO and LNβ-EGFP) into perivitelline space. We injected retroviral vectors into the perivitelline space of in vitro matured bovine oocytes of M II stage. These bovine oocytes were co-cultured with sperm (1×10^6/ml) treated by Percoll gradient for 42~44 hr. Only fertilized embryos were co-cultured with bovine oviduct epithelial cells in 500 μl of CRI aa containing 10% FBS for 8 days. Finally, embryos developed in vitro were examined to evaluate the integration of EGFP and EPO by PCR and the expression of EGFP gene in the blastocyst stage by fluoresence emission. Among embryos microinjected with retroviral vectors, 52.8 (EGFP) and 45.9% (EPO) were cleaved and then 17.3 (EGFP) and 22% (EPO) were developed to the blastocyst stage. Integration of EGFP (386 bp) and EPO (702 bp) in these blastocyst stage were 21.1 and 22.2% by PCR analysis. The green fluorescence was emitted in all EGFP gene integrated blastocysts. Therefore, we demonstrated that reverse-transcribed gene