P-9 Interspecies Nuclear Transfer using Bovine Oocytes Cytoplasm and Somatic Cell Nuclei from Bovine, Human, Porcine and Mouse

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Objective: This study was desined to examine the ability of the bovine (MII) oocytes cytoplasm to support several mitotic cell cycles under the direction of differentiated somatic cell nuclei of bovine, human, porcine and mouse.

Materials and Methods: Bovine GV oocytes were matured in TCM-199 supplemented with 10% FBS. At 22 h after IVM, recipient oocytes were stained by with 5 μg/ml Hoechst and their 1st polar body (PB) and MII plate were removed by enucleation micropipette under UV filter. Ear skin samples were optained by biopsy from an adult bovine, human, porcine and mouse and cultured in DMEM with 10% FBS. Also, individual fibloblast confirmed normal chromosome number in the specificity of species. NT units produced by electrofusion of enucleated bovine oocytes with individual fibroblast. The reconstructed embryos were activated in 5 μM ionomycin for 5 min followed by 1.9 mM 6-dimethylaminopurine (DMAP) for 3 h in CR1aa. And cleaved NT embryos were cultured in CR1aa medium containig 10% FBS under monolayer of bovine cumulus cell.

Results: The cleavage rates were 45.5%, 42.4%, 57.5% and 50.0% from bovine, human, porcine and mouse NT embryos, respectively. Timing of the first two cleavage divisions corresponded more closely to the timing of cleavage observed in bovine in vitro produced embryos regardless of the donor species. Proportion of the morula and blastocyst was 22.2% and 7.1% in NT embryos from bovine and human fibroblast. But NT embryos from porcine and mouse fibroblast are blocked at 16~32-cell stage. In order to determine normal cloned embryo, we checked for chromosome of NT embryos. The number of chromosome in NT embryos from individual fibroblast was the same as chromosome number of individual species.

Conclusion: These results show that bovine MII oocytes cytoplasm has the ability to support several mitotic cell cycles directed by newly introduced nuclear DNA.

P-10 The Study on Vitrification and Ultrarapid Thawing of Human Embryonic Stem Cells

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Objective: This study was carried out to establish the effectiveness of the vitrification method and the