

Interspecies Nuclear Transfer using Bovine Oocytes Cytoplasm and Somatic Cell Nuclei from Various Mammalian Species

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This study was designed 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. Bovine GV oocytes were matured in TCM-199 supplemented with 10% FBS. At 22 h after IVM, denuded recipient oocytes were stained with 5 μ g/ml Hoechst and their 1st polar body (PB) and MII plate were removed by enucleation micropipette under UV filter. Ear skin samples were obtained by biopsy from an adult bovine, human, porcine and mouse and cultured in DMEM with 10% FBS. Also, normal chromosome number in the specificity of species was confirmed in individual fibroblast. NT units were produced by electrofusion of enucleated bovine oocytes with individual fibroblast. The reconstructed embryos were activated in 5 μ M ionomycin for 5 min following 1.9 mM 6-dimethylaminopurine (DMAP) for 3 h in CR1aa medium. And cleaved NT embryos were cultured in CR1aa medium containing 10% FBS under monolayer of bovine cumulus cell. The cleavage rates were 52.7%, 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 blastocysts was 34.5% 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. These results show that bovine MII oocytes cytoplasm has the ability to support several mitotic cell cycles directed by newly introduced nuclear DNA.

Key words) *Interspecies, Nuclear transfer, Bovine oocytes, Cytoplasm*