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

optimal cryoprotectants in the cryopreservation of human embryonic stem cells (ESC).

Materials and Methods: Human ESC clumps established at Seoul National University Hospital (SNUhES 1) were cryopreserved with the vitrification method using the EM grid. EDS and EFS40 were used as vitrification solutions.

EDS step1: DMEM + 10% ethylene glycol + 10% DMSO

step2: DMEM + 20% ethylene glycol + 20% DMSO + 0.5 M sucrose

EFS40: DMEM + 40% ethylene glycol + 18% Ficoll + 0.5 M sucrose

Results: Between the EDS and EFS40 groups, there was no significant difference in the recovery rate after cryopreservation of human ESC. The formation rates of ESC colonies in the vitrified groups were significantly lower than those in the control ESC group ($p<0.05$, $p<0.05$). In addition, the formation rate of ESC colonies in the EDS group was significantly higher than that in the EFS40 group ($p<0.05$). The ESC colonies in the vitrified groups were significantly smaller after culture duration of 2 and 4 days, respectively, compared with the control ESC group ($p<0.01$, $p<0.05$). However, these effects could be reduced to nonsignificant level by the additional culture of ESC colonies. The vitrified human ESC retained the properties of pluripotent cells, including the expression of cell surface markers for the undifferentiated cells such as alkaline phosphatase and SSEA-4 (stage-specific embryonic antigen-4), and the expression of transcription factor Oct-4 (octamer-binding transcription factor-4), and the normal karyotype.

Conclusion: The vitrification method using the EM grid and EDS solution was confirmed to be very effective for the cryopreservation of human ESC.

P-11 Maintenance of Human Embryonic Stem Cells Derived from Frozen-thawed Blastocysts on Feeder-free Culture Condition

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Objective: This study was to confirm whether the established human embryonic stem (hES) cell growth can be maintained without mouse embryonic feeder (MEF) cells.

Materials and Methods: The hES cells (MB02 and MB03) derived from frozen-thawed blastocysts were subcultured until 10th passage (about 2 month, 40 population doublings) on MEF feeder. And then some of these hES colonies were cultured on feeder-free condition using Matrigel-coated plate/STO cell conditioned medium. Characterization of hES cells cultured on feeder or off feeder were taken by alkaline phosphatase staining, karyotyping, cell surface marker staining, Oct4 gene expression and telomerase activity.

Results: The hES cells cultured on feeder-free condition during subculture (about 6 month, 120 population doublings) indicated stable proliferation rate, normal karyotype. high telomerase activity. Similar to cells cultured on feeders, hES cells maintained under feeder-free conditions expressed Oct4, alkaline phosphatase, surface marker (SSEA-4, TRA-1-60, TRA-1-81). Also, embryoid bodies cultured on gelatin dish