B-1 Nuclear Reprogramming in Bovine Oocytes Following Somatic Cell Nuclear Transfer

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Objective: Limited information is available during nuclear reprogramming in nuclear transferred embryos. In this study we determined DNA synthesis and nuclear remodeling following somatic cell nuclear transfer in bovine oocytes.

Method: Matured bovine oocytes were enucleated by aspirating the frist polar body and metaphase chromatin using a beveled pipette. Bovine fibroblast cells were fused into enucleated oocyte by electrical stimulation. Reconstructed oocytes were activated with ionomycine and 6-dimethylaminopurin, and then were cultured in CR1aa medium. The organization of nuclear and microtubules was observed using laser-scanning confocal microscopy. DNA synthesis was labeled with 5-Bromo-2'-Deoxyuridine.

Results: At 1 hour after fusion, microtubule aster was seen near the transferred nucleus in most bovine oocytes. Most of fibroblast nuclei remodeled to premature chromosome condensation (PCC) and to the two masses of chromosome in reconstructed oocytes. Microtubular spindle was seen around condensed chromosome. Gamma-tubulin was detected in the vicinity of condensed chromosome, suggesting this is a transient spindle. The spindle seperated nucleus into two masses of chromatin which developed to two pronuclear like structures. Two pronuclear like structures were than apposed by microtubular aster and formed one syngamy like nuclear structure at 15 h following nuclear transfer. At 17 to 18 h after fusion, two centrosomes were seen near the nucleus, which nucleates micrtubules for two cell cleavage. DNA synthesis was initially detected in reconstructed bovine oocytes at 3 h after fusion and heavily detected in reconstructed bovine oocytes at 6 h after fusion.

Conclusion: These results suggested introduction of foreign centrosome during nuclear transfer, which appeared to give an important role for somatic cell nuclear reprogramming, and DNA synthesis started in nucleus at 3 h following nuclear transfer.

B-2 Establishment and Maintenance of Embryonic Stem Cell and Embryonic Germ Cell in Human and Mouse

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Introduction: Embryonic stem (ES) cells and embryonic germ (EG) cells have an ability to remain undifferentiated and proliferate indefinitely *in vitro* while they have a potency to differentiate all three embryonic germ layers. ES cells are originated from inner cell mass of blastocyst, whereas EG cells are originated from primordial germ cells. ES cells and EG cells were established in almost of mammalian