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Nuclear and Microtubule Reorganization in Cattle Nuclear Transfered Embryos

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Despite of importance of integrated events of nucleus and microtubule remodeling in nuclear transferred embryos with somatic cells, little information is available on this subject. In this study we configured chromatin and microtubule organization following somatic cell nuclear transfer in pre- and non-activated bovine oocytes in order to clearify nuclear remodeling process and to demonstrate centrosome inheritance during nuclear transfer. The cumulus-oocyte complexes were collected from slaughterhouse and were matured in vitro for 20 h in TCM 199 supplemented hormone. 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 cultured in CR1aa medium. The organization of nuclear and microtubules were observed using laser-scanning confocal microscopy. At 1 hour after fusion, microtubule aster was seen near the transferred nucleus in most oocytes regardless activation condition. While most of fibroblast nuclei remodeled to premature chromosome condensation (PCC) and to the two masses of chromosome in non-activated oocytes, a few number of fibloblasts went to PCC and multiple pronuclear like structures in activated 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 the 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. While 31% of reconstructed oocytes in non-activated condition developed to morulae and blastocysts, a few reconstructed oocytes in pre-activated condition developed to the blastocyst. These results suggested introduction of foreign centrosome during nuclear transfer, which appeared to give an important role for somatic cell nuclear reprogramming.

(Key words) ***Nuclear Transfer, Microtubule, Reprogramming, Centrosome***