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Effect of the Timing of Oocyte Activation on Development of Rat Somatic Cell Nuclear Transfer Embryos

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Methods for activation of reconstructed oocytes were examined for the production of nuclear transfer (NT) rat embryos using fetal neural stem cells as donor. Neural stem cells were isolated from Day 14.5 rat fetuses, and the oocytes for recipient cytoplasm were recovered from 4-wk old Sprague Dawley rats. After enucleation and nuclear injection, the reconstructed oocytes were immediately exposed to activation medium consisting of 10 mM $SrCl_2$ for 4 h (immediate activation after injection IAI), or cultured *in vitro* for 2~3 h before activation treatment (injection before activation; IBA). Pre-activated oocytes were also used for NT to avoid spontaneous activation of rat oocytes. The oocytes were grouped as IIA (immediate injection after activation) and ABI (activation 2~3 h before injection). Following NT, the ova were cultured *in vitro*. Development of the NT embryos was monitored at 40 and 115 h of culture. The embryos in groups IAI, IBA, and IIA were cleaved to the 2-cell stage at the rates of 36.6% (15/41), 39.5% (17/43) and 46.3% (25/54), respectively. However, in the ABI group, only one embryo (1.8%, 1/55) was cleaved after activation. After 115 h of *in vitro* culture, two NT embryos from IAI group had developed to the morula/blastocyst stage (4.9%; 2/41) with cell numbers of 8 and 9. However, no morula or blastocyst was obtained in the other groups. These results suggest that immediate activation after injection (IAI) method should be used for the production of rat somatic cell NT embryos.

*This study was supported by Monash University, Melbourne, Australia

Key words) ***Nuclear transfer, Rat, Oocyte activation***