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Porcine Somatic Cell Nuclear Transfer by Whole Cell Intracytoplasmic Injection or Electrofusion

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This study was designed to evaluate the efficiency of whole cell intracytoplasmic injection (WCICI) for production of cloned porcine embryos, bypassing the need for fusion or nucleus isolation procedure thereby simplifying the nuclear transfer methodology. *In vitro* matured porcine oocytes recovered from abattoir derived prepubertal ovaries were enucleated by visualizing under UV after staining with Hoechst 33342 and reconstructed with somatic cell by WCICI (pipette diameter ~10μm) or electrofusion (pipette diameter ~20μm). In the later group, fusion was achieved by single DC pulse of 2.1 KV/cm for 30 μsec. Reconstructed embryos were activated by single DC pulse of 1.5 KV/cm for 30 μsec and exposed to 7.5 μg/ml Cytochalasin B for 3-4 hour before culturing in NCSU23 medium supplemented with 0.4 mg/ml BSA for 7 days at 39℃ in humidified atmosphere of 5% CO_2 in air. Results indicated that, although there were no significant difference in the cleavage rate among the groups, higher percentage ($P<0.05$) of blastocyst were produced by WCICI of ear fibroblast (31.09±8.49%) than electrofusion of cumulus cells (12.52±3.41%) or ear fibroblast (15.32±6.57%). Reprograming time of 2 hour gave better blastocyst rate (31.09±8.49%) than 1 hour (6.89±4.94%) or 4 hour (17.86±11.49%) post-injection in the WCICI group ($P<0.05$). Nuclear swelling and pseudopronucleus formation was apparent at 3 h and 12 h post-activation, respectively. The expanding and hatching ability of cloned blastocyst did not differ among the groups. Therefore, the present study suggests that WCICI can provide an efficient and less labor-intensive method for production of cloned porcine embryos by somatic cell nuclear transfer. This work was supported by a grant from Research project on the Production of Bio-Organs, Ministry of Agriculture and Forestry, Korea.

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