

**Transfer of Porcine Embryos Injected with Sperm
Carrying with Exogenous DNA**

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The main goal of this study was to produce transgenic piglets by the method of injection of sperm-mediated exogenous DNA. Spermatozoa (1×10^6 sperm of final concentration) obtained from caudal epididymis were mixed with pBC1-hEPO (20 ng/ μ l) or pcDNA3 LAC Z (20 ng/ μ l), and followed by electroporation (500 V, 25 μ F). Matured oocytes having the first polar body and dense cytoplasm were selected and centrifuged at 12,000g for 6 min. After sperm injection, the oocytes were activated electrically (1.7 KV/cm, 30 μ sec, single pulse) in 0.3 M mannitol solution. Eggs injected sperm were cultured in NCSU 23 medium (0.4% BSA) at 39°C, 5% CO₂ in air for 192 h. This study were comprised 3 experiments. Experiment 1 compared the developmental efficiencies between the sperm-injected oocytes (Group 1) and further activated electrically (Group 2). Experiment 2 compared the expression of pcDNA3 LAC Z in the embryos produced by Group 1 and Group 2. Finally, experiment 3 carried out transfer of embryos (1-8 cell stage) transfected with pBC1-hEPO into surrogate recipients synchronized by injection of combination of PG600 with hCG. The rates of cleavage and development into blastocyst stage in Group 2 were significantly higher than those of Group 1 (71.3% and 28.1% vs. 43.3% and 10.3%, respectively, P<0.05). Thirty (24.2%) out of 124 embryos analyzed in Group 2 were positive by X-gal. Similarly, in Group 1, 16.3% (8/49) were positive. After transfer of 789 embryos to 7 recipient gilts, three out of them examined by ultrasound became pregnant. One recipient is in day 50 pregnancy. On day 54 of gestation, two were carried out uterotomy in order to confirm the pregnancy. One had 7 and another had 2 fetuses. We conclude that injection of sperm-mediated gene transfer will be used as a valuable tool for the production of transgenic piglets.

(Key words) *exogenous DNA, ICSI, embryo transfer, pig*