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Production of Transgenic Porcine Embryos by Oocyte Mediated Gene Transfer

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Classical approaches for producing transgenic livestock require labor-intensive, time- consuming, and expensive methods but have low transgenic efficiency and high mosaicism rate. This study evaluated a simplified method for producing transgenic porcine embryos by microinjecting DNA construct into unfertilized metaphase oocytes that were subsequently fertilized *in vitro*. For this, oocytes recovered from abattoir derived prepubertal porcine ovaries were matured *in vitro* for 42~44h and were microinjected with DNA solution (10 ng μL^{-1}) using femtojet microinjector (Eppendorf, Hamburg, Germany). The DNA (4.7 kb) was derived from the pEGFP- C1 plasmid (Clontech Laboratories Inc., CA, USA), which contains enhanced green fluorescent protein (EGFP) encoding transgene under the control of cytomegalovirus promoter, and linearized with *Apa*LI restriction enzyme. Injected oocytes were then *in vitro* fertilized using fresh epididymal sperm obtained from abattoir derived porcine testis by standard procedure and cultured in NSCU23 medium supplemented with 0.4% BSA. The efficiency of transgenesis was monitored by visualization of green florescence under UV illumination using EGFP filter set. Data were analyzed by student's *t*-test. Results showed that the cleavage rate of injected oocytes (68.7 ± 0.5%) was similar to those of non-injected control oocytes (67.8 ± 0.4%). However, a high percentage of injected oocytes showed developmental block at 2~4 cell stage. The EGFP expression rate at 2~4 cell stage, when expressed as proportion of injected oocyte, was 17.2 ± 0.1%. Interestingly, mosaicism was not observed. The EGFP expression rate increased to 26.7 ± 0.1% by increasing the DNA concentration to 40 ng μL^{-1}. Injecting the

DNA solution near metaphase plate of the oocyte did not improve ($p<0.05$) the EGFP expression rate (22.2 ± 0.1%). A high proportion of EGFP expressing oocytes blocked at 4~8 cell stage and did not progress to blastocyst suggesting random integration of the transgene in developmentally important gene loci. Our results thus, suggest oocyte mediated gene transfer as a promising tool for producing transgenic livestock. However, further research is required to improve its efficiency.

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