

**Sperm-Mediated Gene Transfer by Injection of Sperm or Sperm Head into Porcine Oocytes**

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The exogenous gene transfer by intracytoplasmic sperm injection (ICSI) procedure has been recently used to produce transgenic mice and pigs. Sperm-mediated DNA transfer has the potential to markedly simplify the generation of transgenic animals. This method may serve as an alternative to the pronucleus injection of DNA for the production of transgenic pigs. Therefore, in this study, we investigated the expression of transgene after co-injection of spermatozoon or sperm head with green fluorescent protein (GFP) gene into *in vitro* matured porcine oocytes. Spermatozoon and sperm head, that was obtained by sonication, were treated with 0.03% Triton X-100 to remove the membrane. They were preincubated with linearized pEGFP-N1 for 1 min, and then embryos cultured NCSU23 medium for 2.5 days after co-injected of sperm and DNA. We monitored expression of GFP in embryos under epifluorescent microscope. The remove of sperm membrane did not alter the developmental competence of embryos after ICSI. At 7 days following injection, the rates of blastocysts following injection of intact sperm (15.0%), and of sperm with disrupted membrane (14.2%) were higher than that following IVF (10.0%). Porcine oocytes injected with sperm which co-cultured with DNA concentration of 1, 0.1, and 0.01 ng were 60, 65.7 and 75% and 18.5, 37.4 and 22.2% for rates of cleavage and GFP expression, respectively. *In vitro* matured porcine oocytes injected with sperm and isolated sperm head resulted in 69 and 59.7% of cleavage rates, respectively. The rates of embryo GFP expressed did not significantly different between sperm (20.4%) and sperm head (20.0%) injection. The transgenic embryos with the clusters of positive blastomeres were observed under fluorescent microscope. Most of embryos expressed GFP gene showed mosaicism. They showed GFP expression at 1/4, 2/4 and 3/4 of blastomeres at the 4-cell stage. Among these 4-cell embryos, the expression rate of 1/4 blastomere group (54.6%) was higher than the other groups (15.3-30.7%). These results indicate that membrane disrupted sperm could attach with exogenous DNA, and that this procedure may be useful to introduce foreign gene into porcine oocytes. Therefore, our data suggest that the ICSI can be a useful tool to efficiently produce transgenic pig as well as other mammals.

**(Key words)** *Exogenous DNA, Sperm, ICSI, GFP, Transgenic pig*