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Expression of GFP Gene in Porcine Embryos after ICSI with Different DNA Binding Methods

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Transgenic animals are produced primarily by microinjecting exogenous DNA into the male pronuclei of a zygote. Microinjection method for gene transmitting is successful in mice but not efficient in farm animals, limiting it's general utility such as a large scale facility and labour. Based on our finding that sperm cells bind with exogenous DNA, sperm was used as a vector for producing transgenic animals to introduced green fluorescence protein(GFP) gene. Expression of exogenous gene in porcine embryos after injection of spermatozoa carring with GFP DNA fragment was investigated. For DNA binding to sperm heads, 0.05% Triton X-100 and DNA/Liposome complex were used and all cultured embryos was activated with Ca-ionophore. The blastocyst rate in the activation groups were significantly higher than that in the non-activated group(36.0% and 28.9% vs 5.1%). Blastocyst rates after sperm mediated gene transfer were no significant difference in DNA/Liposome complex, Triton X-100 and ICSI only groups (18.8%, 19.2% and 25.3%). GFP expression rate in two different DNA binding groups was higher in Triton X-100 group(40.6%) than DNA/Liposome complex group (36.4%), but GFP whole expression rate as no mosaic in blastocyst embryos was higher ($P<0.05$) in DNA/Liposome complex group(4.2%) than Triton X-100 group(0.9%). These results show that sperm mediated gene transfer method should be application to produce transgenic animal, and provides an efficient procedure for studies involving large animal models.

Key Words : ***ICSI, EGFP, Sperm, Porcine, Embryo***