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Positive Expression of EGFP in Bovine Embryos after ICSI using Spermatozoa Co-cultured with Exogenous DNA

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There are many methods to introduce exogenous DNA into embryo for the purpose of producing transgenic animals. Exogenous gene can be integrated into oocyte as a form of sperm vector. In this study, sperm was used as a vector for transgene that is enhanced green fluorescent protein (EGFP). The objective of this study was to investigate the expression of exogenous gene in bovine embryos after injection of spermatozoa cocultured with EGFP fragment. Spermatozoa were plunged into liquid nitrogen and thawed several times or shaked in 0.2% Triton X-100 to remove sperm membrane which followed by DTT treatment. The injected oocytes were co-cultured with vero cells in CR1aa, and expression of EGFP was observed under fluorescent microscope. Blastocyst formation rates of oocytes injected with DTT-treated, DTT-freezing and DTT-Triton X-100 treated sperm were 34.7, 39.4 and 31.9% respectively. EGFP expression rates of oocytes injected with DTT-treated, DTT-freezing and DTT-Triton X-100-treated sperm were 0, 19.1 and 13.9%. On the other hands, expression rate of concentration with 2.5 ng, 5 ng and 12.5 ng were 6.7, 9.0 and 5.1%. When intact sperm was mixed with EGFP fragment, concentration of 12.5 ng/$\mu\ell$, and then electroporated before injection, the expression rate of injected oocyte was 2%. Unexpectedly, electroporation could not increase the expression rate. These results indicate that sperm can be used as a transgene vector, even if the efficent was low (19.1%).

Key words) ***Exogenous DNA, ICSI, EGFP, Bovine, EFGP concentration***