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Production of Transgenic Bovine Embryos by Intracytoplasmic Injection of Sperm Mediated Exogeneous Gene

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Successful attempts of using sperm as a vector to transfer exogenous DNA into eggs have been reported. However, controversies for the success still have been remained. The main purpose of this study, therefore, was performed to clarify the efficiency producing transgenic embryos by use of intracytoplasmic injection of sperm-mediated exogenous DNA.

Spermatozoa selected off by a Percoll-density gradient were treated with/without 5 mM DTT solution for 30 min. After washing, those spermatozoa were incubated in a solution of DNA fragment (50 ng/1105 sperm, mWAP/hGH construct, provided from Dr. M. Nishihara) for 30 min, followed by centrifuging twice in HEPES-TALP at 350 g for 10 min. Oocytes matured in vitro for 22-24 h at 39C in a humidified atmosphere of 5% CO2 in air were stripped of cumulus cells by vortexing in 3% sodium citrate solution. Only oocytes with an extruded first polar body were selected and randomly allocated into two sperm injection groups; being injected with sperm un-treated dithiothreitol (DTT, Group 1) and treated-DTT (Group 2). All oocytes injected single spermatozoa directly into ooplasm were activated with 5 M ionomycin (Ion, 5 min), followed by CDC2 kinase inhibitor (1.9 mM sodium pyrophosphate, 3 h) to prevent the re-accumulation of maturation promoting factor. Differences between treatments were analyzed by ANOVA program after arc-sine transformation of proportional data ($P<0.05$).

In experiment 1, sperm decondensation, pronucleus formation, and subsequent development of oocytes after injection of DTT-treated sperm were compared to those of control group (sperm untreated DTT). Pretreatment of sperm with DTT led to a high pronuclear formation, cleavage and development into later stage than those of control group (56% vs. 20%; 60% vs. 26%; 15% vs. 5%, respectively, $P<0.05$). In experiment 2, those embryos that developed into morula/blastocysts

were assessed for the expression of hGH gene by RT-PCR and Northern dot-blotting. Two out of 15 embryos (13%) were analyzed positive by both of RT-PCR and Northern dot-blotting in DTT-treated group. In contrast, none (0/6) of embryos were positive in control group.

We conclude that pretreatment sperm with DTT enhances pronuclear formation, cleavage and developmental rates. Furthermore, it may facilitates the binding of exogenous DNA into sperm membrane. Supported by Korea Research Foundation (KRF-99-041-G00150G7013).