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Follow-Up of Exogenous DNA by Sperm-Mediated Gene Transfer Via Liposome

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To examine the feasibility of using a sperm vector system for gene transfer, we have investigated the binding and the uptaking of foreign DNA into the sperm nucleus by PCR, *in situ* hybridization and LSC. We have also examined the transportation of exogenous DNA into oocytes by immunofluorescene via PCR. Sperm cells were incubated with DNA/liposome complexes (1 : 4 ratio) in fertilization medium with BSA or without BSA. In situ hybridization demonstrated that the transfection rate of sperm cells with and without BSA was 41 and 68 % respectively, when the cells were treated with liposome/DNA complexes and 13% for DNA alone. LSC analysis showed that the binding of exogenous DNA was greatly reduced by DNase I treatment which digests DNA bound onto spermatozoa, suggesting that some of the DNA was internalized into the sperm membrane. To find out whether transfected DNA was internalized into sperm intracytomembrane, sperm DNA was amplified by inverse PCR. No PCR products were detected from sperm cells, indicating that the foreign DNA was simply bound onto the sperm membrane. To investigate transfer rates of exogenous DNA into oocytes via sperm cells, we used immunofluorescene method to follow the distribution of foreign DNA via spermatozoa: a few exogenous DNA was located in the cytoplasm of early embryos (13/60, 21.7% for DNA+/liposome+/BSA−) and was not located in the pronucleus and/or nucleus. These results suggest that most of the transfected sperm cells could carry the foreign DNA into the egg by in vitro fertilization, but that the transferred DNA is degraded in the developing embryos without stable integration into the zygote genome. Therefore, we have directly injected with transfected sperm cell into oocyte cytoplasm and observed that some of the exogenous DNA was detected in preimplantation embryonic cytoplasm and expressed at preimplantation stages, suggesting that exogenous DNA in early zygote has their integrity. In this study, we have not identified a noble mechanism that interfering transportation of foreign DNA into zygote genome via spermatozoa. Our data, however, demonstrated that inverse PCR and immunofluorescene methods would be used as a new tool for follow-up of gene distribution in oocyte via sperm cells.

Key words) ***sperm-mediated gene transfer, liposome, LSC, in situ hybridization, ICSI***