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Uptake of exogenous DNA by using liposome in boar spermatozoa

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This study was based on the ability of sperm cells to bind and internalize exogenous DNA and to transfer it into oocytes at fertilization. The aim of this study was to examine uptake of exogenous DNA by using liposome in boar spermatozoa. Mitochondrial DNA (mtDNA) of chicken's liver tissue for exogenous DNA were isolated and purified by the alkaline lysis method. After mtDNA was digested with EcoR I and *Hind* Ⅲ, 1.2kb DNA fragment was obtained from a low-melting agar gel. 736㎕ of Androhep(-) extender without BSA and antibiotics was mixed with 40㎕ of liposome (Sigma) solution at room temperature for 5 min and then added 24㎕ of exogenous DNA (40ng/㎕), incubated at room temperature for 20 min. After spermatozoa were washed twice in Andreop(-) by centrifuging at 5000 rpm for 5 min, the pellet mixed with previous prepared exogenous DNA / liposome complex at 17℃. Estimation of quantity of exogenous DNA was conducted at 10, 20, 30, 60, 90 and 120 min of incubation. After incubation, 10^6 spermatozoa was centrifuged twice at 3000 rpm for 5 min, the pellet was treated with DNase I(5U/㎕) 37℃ for 1 h. For PCR analysis, nuclei of 10^6 spermatozoa was isolated using the alkaline lysis method. Boar spermatozoa DNA was used as a negative control and plasmid DNA of a 1.2kb DNA fragment was used as a positive control. When liposome was used, internalization of exogenous DNA in sperm cytoplasm was more efficient than use of exogenous DNA alone. The internalization of spermatozoa-bound DNA was completed within 60 min at 17℃. In summary, the use of liposomes as vector to introduce exogenous DNA in boar spermatozoa could be a useful method to generate and investigate the transgenic animals.

Keywords: ***liposome, boar spermatozoa, exogenous DNA, DNA Uptake***