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Development of In Vitro Porcine Oocytes Following Intracytoplasmic Injection of Sperm-Mediated GFP Gene

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Transgenic animals production tools have been valuable for research and purpose. The current methods of gene transfer, microinjection and nuclear transfer, which are widely used in transgenic animal production, but all most methods has only had limited success in production of larger species. Here, we report the possibility of a sperm-mediated gene transfer method in porcine embryos. Oocytes were collected from ovaries harvested at a local slaughterhouse were matured in 500㎕ drops of TCM-199 under mineral oil at 38.5℃ in a humidified atmosphere of 5%CO2 in air. After 42-43h of in vitro maturation oocytes were denuded. For sperm injection into the cytoplasm of the porcine oocytes, sperm suspension in NIM medium are subjected extraction with TritonX-100 before mixing with a green fluorescent gene (GFP). Sperm with TritonX-100 were prepared by adding TritonX-100 to a final volume of 0.05% in the sperm suspension and mixing by trituration for 60s before two wishes in NIM medium at 2℃. After wishing, sperm were mixed with TritonX-100 at 25℃ followed by washes at 2℃. Sperm were resuspended in ice cold NIM to a final volume of 400㎕ and 2-20ng/㎕ DNA were triturated on ice for 60s. All microinjection was performed in HEPES-buffered CZB medium at room temperature within 2h. After culture in NCSU-23 for 72h, percent of porcine embryos transfected GFP gene are 20.7%(6/29) in 20ng/㎕ sperm-DNA mixed group and other groups were 3.7 %(2/54)and 4.7%(3/67). These data suggests that sperm-mediated gene transfer method should be used to the production tool of transgenic pig efficiently.

Key words) ***sperm, GFP, microinjection, transgenic, porcine***