P-12

In Vitro Embryo Development by Loaded Boar Spermatozoa with Exogenous DNA

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Until now, the Transgenic animals have been producted by genetical biotechnology using 1) DNA microinjection, 2) Nuclear Transfer, 3) viral-based construct. However, these techniques have some problems such as embryo handing, expensive experiment and limited oocyte from donor. Therefore, it has been proposed a development of a simpler and economical transgenic animal production method. This study did use ability that sperm cells are bound and internalized with the exogenous DNA *in vitro*. It has been analyzed quality of loaded boar spermatozoa with exogenous DNA and development of embryo *in vitro* in this study by loaded spermatozoa with circular or linear plasmid, respectively. After spermatozoa were incubated with either DNA solution or liposome/DNA complex for 90 min at 17℃, the viability (SYBR-14/PI) and motility (Makler counting chamber) were analyzed during *in vitro* incubation (0, 2, 4 and 6 hr), and the spermatozoa were used to *in vitro* fertilization. Oocyte matured in TCM-199 medium for 44 hr was fertilized in mTBM for 6 hr. The development of embryo cultured in PZM-3 was assessed at Day 2 and 7 of culture. Exogenous DNA in blastocyst-stage embryo was detected by PCR analysis. Boar spermatozoa extended was used to fresh group and control group was followed by transfection procedure without DNA and liposome. The viability in fresh spermatozoa (83.3 ± 1.7 %) was significantly ($p<0.05$) higher than in control, DNA solution

and liposome/DNA complex groups (72.3 ± 0.2, 70.8 ± 1.8 and 68.0 ± 2.2 %, respectively) at 0 hr of storage. Both viability and motility of all groups after 4 hr of storage were significantly ($p<0.05$) lower than at 0 and 2 hr. On the other hand, development rate (22.2 ± 0.6%) to blastocyst stage of embryo in control group was significantly ($p<0.05$) higher than those of embryo by circular (DNA solution, 9.1 ± 1.3% and liposome/DNA complex, 11.3 ± 0.8%) or linear plasmid groups (DNA solution, 5.9 ± 3.0% and liposome/DNA complex, 5.3 ± 2.7%). However, there were no significant differences on embryo development between loaded spermatozoa using circular or linear plasmid. Additionally, transfection rate of embryo using spermatozoa loaded with circular plasmid was higher than that using spermatozoa loaded linear plasmid. Transfection rates of embryo using liposome in circular (57.3 ± 10.59%) or linear plasmid (48.3 ± 10.63%) was higher then that those using circular (37.8 ± 5.43%) or linear plasmid (34.9 ± 12.60%) alone. These finding raises the possibility that loaded spermatozoa with exogenous DNA could be used to *in vitro* fertilization, and transferred the exogenous DNA into oocytes.

Key words) *Loaded spermatozoa, Circular and linear plasmid, In vitro Development, Ransfection*

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