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Efficiency of Transgenesis by Using Sperm Mediated Gene Transfer on the Cultured Prepubertal Mouse Testicular Cells

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Exogeneous DNA can reproducibly be delivered by co-injected spermatozoa and this transgenesis method is very efficient protocol. But, mosaic patterns of transgenic embryos and offspring were shown frequently. Whole blastomere integration is important in transgenic animal economics. This study was performed to evaluate the whole blastomere integration of exogeneous DNA in each blastomere and to improve the transgenic efficiency for sperm mediated gene transfer technique. BDF1 mouse oocytes were piezo-pulse assisted microinjected with different sperm source for the production of exogenous eGFP DNA harbouring embryos. Three groups were divided by sperm source. First group were used by frozen thawed sperm head co-incubated with linear eGFP DNA for binding. Second group were used *in vitro* cultured sperm from 14~16 day old ICR mouse testicular cells for 2 weeks. For the introduction of eGFP DNA into testicular cells, electroporation was excuted before the culture. Spermatogenic cell culture was performed under DMEM/F12 basal medium with rFSH, testosterone, GM-CSF, SCF, ITS and retinoic acid. As a control, fresh sperm were used to compare developmental rate in each group. The expression of eGFP protein was confirmed under epifluorescence microscopy. At 8-cell stage, number of eGFP expressing blastomere were evaluated. To evaluate mRNA transcript expression and chromosomal integration in each blastomere, single blastomere RT-PCR and FISH were excuted in each blastomere at 8-cell stage embryos.

PN formation, cleavage and blastocyst formation rates were not significantly different in each group and eGFP expression rate was not different, respectively. But, in each blastomere evaluation at 8-cell stage embryos, expression features were different. None of embryos derived by co-incubation group showed whole blastomere expression pattern. But, embryos derived by *in vitro* cultured sperm showed whole expression patterns.

Introduction of exogeneous DNA into pre-meiotic spermatogenic cells improves DNA integration in the mouse whole embryos. Therefore, *in vitro* spermatogenic cell culture would be efficient in spermatogenic cell mediated gene transfer.

Development of mouse embryos by *in vitro* cultured sperm injection

	No. oocytes	No. embryos (%)					
		PN	Cleaved	4~8	M	BL	GFP(+)
Control	244	160(65.6)	120	115	109	68(56.6)	-
Co-incubation	212	129(60.8)	97	90	85	47(48.5)	15(31.9)
In-vitro cultured sperm	190	112(58.9)	85	82	77	43(50.5)	17(39.5)

Key words: ***Sperm mediated gene transfer, Mosaic patterns, Spermatogenic cell culture, Single blastomere RT-PCR, FISH***