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Expression of Stage-Specific Genes on the Cultured Spermatogenic Cells Obtained from Prepubertal Porcine Testis

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Achieving of *in vitro* development for mammalian premature spermatogenic cells are very difficult. *In-vitro* culture of spermatogenic cells were then initiated in an effort to try to study *in vivo* spermatogenesis and to understand its molecular events. Recently, the morphogenetic changes of spermatocytes or spermatid by *in-vitro* culture system were achieved. Also, the techniques of spermatogenic cell mediated gene transfer for transgenic animal production technology were advanced. In example, auto/xenotransplantation of spermatogonial cells and sperm mediated gene transfer by using linker proteins were developed. The establishment of spermatogenesis *in vitro* is very important for understanding of molcular events in male reproductive organ and for utilizing of transgenic animal production. Therefore, we evaluated the differentiating ability during *in vitro* spermatogenic cell co-culture system estimated by observation of morphogenetic modifications and by comparing of spermatogenic stage specific gene transcript expression levels. and we determined the integration of exogeneous gene through the introducing marker eGFP gene.

49~68 day old prepubertal porcine testes were enrolled. enzyme treated single spermatogenic cells were co-cultured with empty zona pellucidae on TM4 Sertoli cell monolayer at 33℃, 5% CO_2 in air. Survival rates were compared by trypan blue exclusion test and Hoechst staining. In order to estimate the differentiation on the culture days, RT-PCR for each stage specific expressed genes (Transition protein 2 (TP2), Sperm protein 38 (Sp38)) was performed.

The survival rate in encapsulated system attenuated the time-dependent death rate than classic culture system. The survival rate of spermatogenic cells was gradually lower proceeding the culture periods. Comparing the mRNA expression levels, TP2 expression was first detected on culture day 3 and Sp38 was detected on day 6. Both were slightly increased on the culture days.

The encapsulation of spermatogenic cells with zona pellucidae in co-culture system enhanced cell survival and differentiation rates by protecting from loss of germ cells and various damage during *in-vitro* culture. also, It is suggest that transgene contained spermatozoa is able to produce through the short term culture of prepubertal stage porcine male germ cells.

Key words) ***spermatogenic, co-culture, prepubertal, stage specific, transgenic***