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 molecular 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°C, 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