Results: The proportion of oocytes with NEBD significantly increased in groups with over 2 hours exposure time ($p<0.05$). The proportion of eggs with PCC rate at 4 hours increased significantly compared to that others (25.0% vs 0~13.3%) post injection ($p<0.05$). Normal fertilization and development rate to the blastocyst stage were significantly higher in post (61.8% and 19.7%) activation group compared to that of pre (32.4% and 10.9%)or immediate (39.4% and 9.1%) activation groups ($p<0.05$).

Conclusions: The optimal exposure time of peak NEBD rate is 2~3 h after injection. And, activation in 2~3 hours after round spermatid injection improved the normal fertilization and early embryo development rate.

O-5 Effect of the Some Neural Inducing Factors on Neural Differentiation of Human Embryonic Stem Cells

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Background & Objectives: Embryonic stem cell has pluripotent and self-renewal characteristics, so it is expected that used for clinical application as a source of a cell therapy for variable degenerative diseases. Because ES cells have unlimited proliferation capacity, neural progenitor or terminally differentiated neural cells derived from ES cells might be used for alternative neural cell source. In this study, we test some inducing factors for finding of optimal condition of neural differentiation of human ES cells in vitro.

Method: Human ES cell lines, SNUhES-1, SNUhES-2, SNUhES-3, were used for neural differentiation. Undifferentiated ES cells were cultured on a feeder layer of mitomycin-C treated mouse embryonic fibroblasts. ES cell colonies were mechanically separated to proper size containing about 500~800 cells at 5 days after the transfer. These dissociated colonies were transferred into bacterialogical culture dish containing ES cell culture medium without bFGF for embryoid body (EB) formation. After the 4-day culture, EBs were cultured for 8~9 days on gelatin and laminine coated culture dishes or cover slips in EB media containing N2 supplements, 20% SR or FBS, with or without the differentiation factors, bFGF, LIF, RA, β-NGF, for neural progenitor cell enrichment. Whole or some parts of nestin-enriched EB forming the neural progenitor network were separated mechanically and cultured for one day in suspension condition to form the neurosphere-like structure. They were transferred onto fibronectin coated culture dishes and cultured with additional differentiation factors, RA, β-NGF, EGF, PDGF, each during 2~3 weeks. Nestin enriched EBs and further differentiated cells from human ES Cells were confirmed by flowcytometry, RT-PCR and Immunofluorescence using specific Abs against variable markers of neural progenitor (nestin), early or mature neuron (N-CAM, neurofilament, betaIII tubulin), glial cell (GFAP) and Fluorescence conjugated secondary Abs.

Results: ES cells derived neural progenitors expressed the nestin were more increased by bFGF and LIF addition in EB media containing N2 supplements than control culture condition. Nestin mRNA expressions were increased time dependently but after the 8-day culture, they were decreased rapidly. After the neural

progenitor enrichment culture, N-CAM was expressed in most cells. bFGF, strong mitogen for neuroepithelial precursor cells, used for proliferation of neural precursor cells derived from human and mouse embryonic stem cells. LIF is known to that inhibits the formation of cardiac mesoderm and promote the neuronal differentiation at early stages of CNS development or differentiation of mouse ES cells. Our result suggests that bFGF and LIF effectively promoted the neural progenitor differentiation in human ES cells also. RA affected to differentiation of another cell types as well as neuronal cells and β-NGF more effectively induced the neuronal differentiation.

Conclusions: In this study, neural progenitors and further differentiated neuronal cells could be induced from human ES cells and every differentiation stage could be controlled by differentiation times and some inducing factors. It was expected that neural progenitor and differentiated cell populations are used for source of transplantation without teratoma formation but it was remained to problem that gained homogeneous cell population. Now, we try to sort the differentiated cells each and apply them to animal model. This research was supported by a grant (SC 11011) from Stem Cell Research Center of the 21st. Century Frontier Research Program funded by the Ministry of Science & Technology, Republic of Korea.

O-6 생쥐 정원줄기세포의 체외배양기간 동안 LIF/bFGF와 FSH에 의한 분화와 증식의 조절

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Background & Objectives: 최근 생식세포의 re-encapitulation이라는 3차원적 체외배양 방법에 의하여 남성불임의 생체 내 기작에 대한 기초과학적 연구가 급속히 촉진되었다. 본 연구소에서는 그 동안 소의 정소내 세포들의 분리한 후 다시 재결합하여 hydrogel의 일종인 calcium alginate를 이용, 새로운 capsule형태로 하여 장기간 배양을 진행하였으며, 그 결과 반수체인 정세포에서 특이적으로 나타나는 유전자의 발현을 관찰할 수 있었다 (Biol Reprod 65: 873-8, 2001). 그러나 지금까지의 배양방법으로는 정원줄기세포로 재증식하지 않는다고 생각되며, 극소수의 정세포만을 관찰할 수 있었다. 따라서 본 연구에서는 생식세포의 증식과 분화에 관여한다고 알려진 LIF/bFGF와 FSH를 3차원적 체외배양법 적용하였을 경우 어떠한 영향을 주는지 알아보고자 하였으며, 이를 통한 정자형성과정의 새로운 체외배양법을 확립하고자 하였다.

Method: 실험재료는 3~5일령의 ICR 수컷 생쥐를 사용하였으며 생쥐정원세포의 세포분리 및 재 결합을 하여 calcium alginate을 이용하여 capsule형태로 만들어 체외배양 하였으며 LIF/bFGF와 FSH를 처리하여 2주간 배양 후 역전사중합효소반응과 면역조직화학법을 이용하여 발현정도와 발현부위를 관찰하였다.

Results: LIF/bFGF를 처리하여 체외배양한 그룹에서 정원세포의 지표유전자와 항체인 Oct-4 mRNA와 Integrin beta1가 유의하게 높게 발현되었으며, 이와는 반대로 FSH처리군에서는 농도의존적으로 감