embryo bodies (EB: for 5 days, without mitogen) from hES cells and then neurospheres (for 7~10 days, 20 ng/ml of bFGF added N2 medium) from EB. And then for the differentiation into neuronal cells, neurospheres were cultured in N2 medium (without bFGF), supplemented with brain derived neurotrophic factor (BDNF, 5 ng/ml) or platelet derived growth factor (PDGF, 20 ng/ml) for 1 or 2 weeks. Specific neuronal cell differentiation was identified with immunocytochemistry using glutamate (1:1000; Sigma), enzyme tyrosine hydroxylase (1:1000; Sigma), serotonin (1:1000; Sigma) and GABA (1:1000; Sigma) antibodies.

Results: In the presence of BDNF or PDGF, most of neural cells derived from hES cells were differentiated into glutamatic and GABA neuron in vitro. But, we confirmed that there are a few serotonin and tyrosine hydroxylase positive neuron in the same culture environment.

Conclusion: This result suggested that most of neural cells derived from hES cells were in vitro differentiated into glutamatic and GABA neuron in the presence of BDNF or PDGF.

P-17 In vitro Development of Somatic Cell Nuclear Transferred Bovine Embryos Following Vitrification-thawing of Enucleated Oocytes at Matured (MII) Stages

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Objective: This study was to evaluate the in vitro survival of vitrified-thawed bovine enucleated MII (eMII) oocytes by minimun volume cooling (MVC) method and their in vitro development after somatic cell nuclear transfer (SONT).

Materials and Methods: Bovine oocytes were recovered from slaughtered bovine ovary and matured in TCM-199 supplemented with 10% FBS. After incubation for 20 h in IVM medium, recipient oocytes were stained using 5 μg/ml Hoechst and their 1st polar body and MII plate were removed by enucleation micropipette under UV filter. eMII oocytes were subjected to activation before (pre-activation group) or after (post-activation group) vitrification in 5 μM ionomycine added CR1aa medium for 5 min. For vitrification, eMII oocytes were pretreated in EG10 for 5 min, exposed in EG30 for 30 sec. Thawing was taken by 4-step procedures [1.0 M sucrose (MS), 0.5 MS, 0.25 MS, and 0.125 MS added PBS, for 1 min per each step] at 37℃. Survived eMII oocytes were subjected to nuclear transfer with cultured adult bovine ear cells. Reconstrusted oocytes were cultured in 10 μg/ml of cycloheximide and 2.5 μg/ml of cytochalasin D added CR1aa medium for 1 h, and then in 10 μg/ml of cycloheximide added CR1aa medium for 4 h. Subsequently, the reconstructed oocytes were washed three times and incubated in CRIaa medium for 48 h. The cleaved embryos were then selected and further cultured on cumulus-cell monolayer drop in CRIaa supplemented with 10% FBS for 7 days.

Results: Survival rates of bovine vitrified-thawed eMII oocytes in pre-activation and post-activation groups were 88% and 80%, respectively. Nuclear transferred eMII oocytes in post-ativation group indicated

significantly higher rates of cytoplasmic fragment than those in pre-activation group. Fusion rates of cytoplasts and oocytes in preactivation group or normal NT group resulted in 67% and 78%, respectively. Also, their subsequent development into cleaved embryos (55% vs. 57%) and blastocysts (7% vs. 15%), respectively.

Conclusion: This result suggested that MVC method was appropriate freezing method for the bovine eMII oocytes and that vitrified eMII oocytes after pre-activation can support in vitro embryonic development after SONT as equally well as fresh oocytes.

P-18 Effects of Growth Factor on the Spermatogenic Cells from Infertile Men in co-culture System with TM4 Monolayer

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Objectives: The development of assited reproduction technique significantly improved pregnancy rate in male infertility. However, the problem of severe idiopathic male infertility were not solved inspite of many efforts. *In vitro* culture of spermatogenic cells were then initiated in an effort to try to overcome the low clinical outcome. Recently, the production of flagellar growing spermatid by *in vitro* culture system was achieved. Also, auto/xenotransplantation technique of spermatogonial cell were developed. The establishment of spermatogenesis *in vitro* is very important for clinical outcome and understanding of molcular events in male reproductive organ. Therefore, we evaluated the effect of growth factors (Stem Cell Factor (SCF), Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF)) in spermatogenic cell co-culture system and estimate for proliferative abilty of encasulation culture system with Sertoli cell monolayer.

Materials & Methods: From Jan. until Oct. 2001, 19 obstructive azoospermic patients and 4 non-obstructive azoospermic patients entering IVF/ICSI programme were enrolled. Spermatogenic cells were co-cultured with or without empty zona pellucidae on TM4 Sertoli cell monolayer supplemented with different concentrations of SCF and GM-CSF up to 168 hrs. Different survival rates were compared by trypan blue exclusion test and Hoechst staining. In order to compare the proliferation and differentiation abilty in different conditions, immunocytochemical staining with anti-*c-kit* antibody was performed. The presence of early spermatogenic cell was confirmed by RT-PCR with *c-kit* primer.

Results: The survival rate in encapsulated system attenuated the time-dependent death rate than free culture system. The survival rate of spermatogenic cells from obstructive azoospermic patients were slightly higher than that of non-obstructive azoospermic patients at 168 hrs culture. Addition of growth factor in encapsulated co-culture system increased proliferation rates of spermatogenic cells. In this system, the most optimal concentrations of SCF, GM-CSF were 1 ng/ml, 1 ng/ml, respectly. Presence of early spermatogenic cells was confirmed by expression of *c-kit* transcript during all culture period.