

있는 아기를 분만하였으나 곧 사망한 경험이 있는 환자의 경우 8개의 배아에서 blastomere를 각각 생검하여 TelVysion 9p와 CepX, Y를 이용하여 FISH로 분석하였다.

정상 또는 balanced carrier로 판정된 3개의 배아를 자궁내 이식하여 현재 정상적으로 임신이 진행 중이며 양수세포를 이용한 FISH 분석과 배양한 세포의 염색체 핵형 검사 결과 46,XX로 판명되었다.

계속되는 자연유산으로 (5회) 임신을 할 수 없었던 환자의 경우 그 핵형이 46,XX, t(12;13) (q15;q12)이었다. 6개의 배아에서 blastomere를 생검하여 TelVysion 12q probe와 CepX, Y를 이용하여 FISH 분석한 결과 모두 비정상적인 배아로 판정되어 자궁내 이식은 시행하지 아니 하였고 계속 배양하여 이 배아들이 모두 난할이 정지된 것을 확인하였다.

**결 과:** 부인이 46,XX, t(9;14) (p22;q31)인 환자에서 착상 전 배아 유전진단 후 정상적인 배아를 선택하여 자궁내 이식하여 현재 정상 염색체를 가진 아기를 임신 중에 있다.

다른 46,XX, t(12;13) (q15;q12) 환자의 경우 6개의 배아 모두가 착상 전 유전진단 후에 비정상 배아였으므로 배아를 자궁내 이식을 할 수 없었다.

**결 론:** 본 연구에서는 translocation carrier의 착상 전 유전진단을 위해 비교적 손쉽게 구입하여 사용할 수 있는 telomeric probe와 CepX, Y를 이용하여 translocation carrier 부부에게 착상 전 유전진단을 시행한 후 정상 임신 1례를 경험하였기에 보고하고자 한다.

## P-23 *In vitro* Differentiation and Survival of Neural Cell Type from Human Embryonic Stem Cell Derived from Frozen-thawed Blastocysts

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**Objectives:** This study was to investigate the neural cell differentiation *in vitro* from the human embryonic stem (hES) cells derived from frozen-thawed blastocyst stage embryo.

**Materials and Methods:** To induce the neural cell and *in vitro* differentiation from hES colony, the neurotrophic growth factors containing EGF, bFGF, PDGF, retinoic acid and NGF were added to the cells. The cell survival rate detected by MTT [3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide] assay and cell counting. Immunocytochemistry, RT-PCR and western blotting were used for identification of neuronal and supporting cells differentiation.

**Result:** In cell counting, the addition of bFGF and PDGF on hES cell increased up to 3 fold than non-treatment group. The cell survival rate detected by MTT assay showed that bFGF increased cell survival rate of 1.33 fold compared to non-treatment group. Especially, PDGF increased cell survival rate of 1.73 fold compared to non-treatment group. Besides neural cell, glia cells were differentiated from hES cell in the presence of bFGF or PDGF. In immunocytochemistry, the neuron was detected with NF160, synapsin and  $\beta$ -tubulin, astrocyte with GFAP, oligodendrocyte with O4, CNPase, S-100 $\beta$  and  $\alpha$ Galactocerebroside, and glial precursor with A2B5. In western blotting, N-CAM was detected as neuron type marker. In the RT-PCR analysis, differentiated hES had neurofilament 200 (NF200) for neuron positive.

**Conclusions:** This study showed that the human embryonic stem (hES) cells derived from frozen-thawed

blastocyst stage embryos were differentiated into neural or glial cell types by specific growth factors. Especially, PDGF and bFGF have an effect on neuronal differentiation and survival than other neuronal growth factors.

## **P-24** Effect on Development of Mouse Preimplantation Embryos in Two Culture Media with Different Compositions of Energy Sources in vitro Culture

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**Objectives:** This study was conducted to examine the effect on development of mouse preimplantation embryos in vitro culture in four culture systems by two media with different composition of energy sources.

**Materials and Methods:** Mouse 2-cell embryos collected from ICR female mice (4~5 weeks) were cultured for 96 hours in vitro. Two-cell embryos of 271 were cultured in different four culture groups by two media with different composition of energy sources (DMEM-G: DMEM with L-glutamine, without D-glucose and sodium pyruvate; DMEM-GGP: DMEM with L-glutamine, D-glucose, sodium pyruvate); Group I (n=61): embryos cultured for 48 hours in DMEM-G and then transferred to fresh same medium, Group II (n=64): embryos cultured for 48 hours in DMEM-GGP and then transferred to fresh same medium, Group III (n=72): embryos cultured for 48 hours in DMEM-G and then transferred to fresh DMEM-GGP, Group IV (n=74): embryos cultured for 48 hours in DMEM-GGP and then transferred to fresh DMEM-G. All experimental media were added to 10% human follicular fluid (hFF). Development of embryos in each group was observed every 24 hours. Results between different groups were analyzed using a Chi-square test, and considered statistically significant when p value was less than 0.05.

**Results:** After 24 hours in vitro culture, the rate of development into  $\geq$  3-cell was significantly higher ( $p<0.05$ ) in Group II (87.5%) and IV (86.5%) compare with Group I (59.0%) and III (62.5%). After 48 hours, the rate of development into  $\geq$  morula was significantly higher ( $p<0.05$ ) in Group II and IV (79.7%) (86.5%) compare with Group I (34.4%) and III (37.5%). However, the developmental rate into blastocyst were not significantly between experimental groups. After 72 hours, the rate of development into blastocyst in Group IV (74.3%) was significantly higher ( $p<0.05$ ) than Group I (49.2%) and Group III (45.8%), but Group IV was not significant ( $p=0.0593$ ) compare with Group II (59.4%). After 96 hours, the rate of development into  $\geq$  expanded blastocyst was significantly higher ( $p<0.05$ ) in Group IV (70.3%) compare with Group I (32.8%), Group II (53.1%), and Group III (40.3%).

**Conclusions:** In conclusion, mouse 2-cell preimplantation embryos development was most effective in culture system with DMEM-GGP for 48 hours and then transferred to fresh DMEM-G.

**Key Words:** Mouse 2-cell embryo, Blastocyst, DMEM, Glucose, Pyruvate