P-13 생쥐 배아의 완만동결과 초자화동결의 비교 연구

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Background & Objectives: 본 연구에서는 체외배양에 의해 얻어진 발달단계별 생쥐 배아를 배아의 동결에 주로 이용되고 있는 완만동결 방법과 초자화동결 방법으로 동결-융해한 후 각각의 동결방법에 따른 배아의 생존율과 발달율을 비교하여 인간 배아 동결보존의 기초 자료로 활용하고자 한다.

Method: 생후 5~8주된 F1 hybrid 생쥐 (C57BL♀XCBA♂) 암컷과 10주 이상된 생식능력 있는 수컷을 사용하여 과배란 유도 후 체외수정을 시행하였다. 수정 후 9시간 후에 전핵배아, 24시간 후 2세포기, 48시간 후 4세포기, 63시간 후 8세포기, 72시간 후 상실배, 96시간 후 포배기의 배아를 얻어 완만동결 (1.5 M PROH + 0.1 M sucrose)과 초자화동결 I (1.5 M & 5.5 M EG + 1.0 M sucrose), 초자화동결 II (10% DMSO + 10%EG & 20%DMSO + 20%EG + 0.5M sucrose)에 따른 생존율과 발달율을 비교 조사하였다.

Results: 냉동 보존된 각 시기 배아들의 동결-융해 후 생존율은 발달단계별 동결방법에 유의한 차이가 없었다. 그러나 동결-융해 후 발달율은 4세포기에서는 완만동결 방법이 초자화동결 I, II의 방법보다 유의하게 높게 나타났으며 (80.5, 69, 69.5%, $p<0.05$) 그 외 전핵기 (66, 96.6, 97.1%, $p<0.05$), 2세포기 (72.6, 88.8, 85.2%, $p<0.05$), 8세포기 (62.9, 84.5, 74.2%, $p<0.05$), 상실배 시기 (69.8, 84.4, 79.9%, $p<0.05$)에서는 완만동결 방법보다 초자화동결 I, II의 방법에서 높게 나타났다. 한편 포배기 시기에서는 완만동결과 초자화동결 II의 방법에서 초자화동결 I의 방법보다 유의하게 높은 발달율을 나타냈다 (36, 41.8, 13.2%, $p<0.05$).

Conclusions: 본 실험에서는 생쥐 배아의 동결-융해 후의 발달율이 발달단계, 동결방법에 따라 다르게 나타났다. 이에 인간 배아의 동결 시에도 발달단계에 따라 적절한 동결 방법을 선택함으로써 동결-융해 후 배아의 높은 생존율과 발달율을 얻을 수 있을 것으로 사료된다.

P-14 Generation of Dopaminergic Neurons from Human Embryonic Stem (hES) Cells by Co-culture with Bone Marrow Stromal Cells

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Background & Objectives: Dopaminergic neuron differentiation derived from the hES cells will be useful source for the Parkinson's disease cell therapy. This study was to evaluate whether the hES cells can be efficiently differentiated into midbrain dopaminergic neuron by co-culture with human bone marrow

stromal (hBMSC) cells.

Method: For neuron differentiation, MB01 hES cells recovered from enzyme treatment were plated on the hBMSC cells in 15% SR supplemented DMEM/F12 (15% SR) medium. At the beginning with neural rosette formation, 15% SR medium was changed in N2 medium containing SHH and FGF8 (P0). After 2 weeks, a number of rosette structures were harvested and replated onto polyorinthine/laminin coated culture dishes in SHH, FGF8, BDNF and ascorbic acid added N2 medium (P1). Gene expression related dopaminergic neuron was analysed in undifferentiated hES cells, P0 (4 wks, P0) and P1 (5 wks) cells using RT-PCR.

Results: Being processed differentiation, undifferentiated ES cell marker including Oct4 and Nanog was gradually reduced and completely disappeared in final P1 cells. We confirmed all kinds of dopaminergic neuron gene expression was presented in even neural rosettes (P0) and differentiated P1 cells (Pax2 and Pax6 as signal induction marker; Ptx3, Nurr1 and En1 as fate determining transcription factor; TH, AADC, DAT as midbrain dopaminergic neuron marker). This fast expression is very efficient to dopaminergic cell production. Interestingly, TH expression was first detected at 23 days after co-culture. However, there was not detected non-dopaminergic cell marker DBH in this culture method.

Conclusions: The generation of dopaminergic neurons from hES cells can be easy, fast and efficiently carried out by co-culture with h-BMSC cells.

P-15 Abnormal Protein Expression Profiles in Human Follicular Fluid from Recurrent Spontaneous Abortion (RSA) Patients

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Background & Objectives: The cellular processes of immunological, metabolic, vascular and endocrine regulation are required for maintaining normal pregnancy. Aberration of these regulating processes may lead to a number of problems in pregnancy including recurrent spontaneous abortion (RSA). RSA, defined as three or more clinical pregnancy losses before the 20th week of gestation, occurs in ~2-5% of pregnant women. This pregnancy loss is the most common complication of pregnancy, as ~10-15% of human conception terminates in a clinically detected spontaneous abortion. However, the specific genes and proteins involved in this problem are not well defined. In order to prepare for more intensive study in the identification of the proteins that are involved in RSA, we established and optimized two-dimensional (2-D) polyacrylamide gel electrophoresis (PAGE) for comparative analysis of the protein expression profiles between normal women and women with RSA.

Method: Human follicular fluids of ovary from normal women and women with RSA were obtained from mature follicules after oocyte collection for in vitro fertilization (IVF). Follicular fluids were cen-