Results: 동결-융해된 배아의 생존율은 71.2±29.8% (mean ± SD)였다. FISH 또는 PCR 분석을 통해 진단이 가능했던 경우는 동결-융해 배아에서는 88.1±22.0%에서, 일반 배아에서는 95.2±10.3%으로 유의한 차이는 없었다. 이식할 수 있는 정상 배아의 비율 역시 두 군간에 차이가 없었다 (21.6±19.7% vs. 27.4±18.8%; $p>0.05$). 할구세포 생검 후 발달이 잘 진행된 배아의 비율은 동결-융해 군에서 62.0± 26.5%, 일반 군에서 66.4±26.1%로 두 군간에 유의한 차이는 없었다 ($p>0.05$). 이식 주기당 임상적 임신율은 동결-융해 군에서는 11.4% (4/35)였으며, 신선배아를 이용한 일반 군에서 21.3% (57/267)로 통계적으로 유의하지는 않았으나 낮은 경향을 보였다.

Conclusions: 본 연구의 결과로 동결-융해된 배아에서의 할구세포 생검 및 이를 이용한 FISH/PCR 분석은 융해된 배아가 잘 생존한다면 신선한 배아에서와 차이 없이 실효성이 있으며, 할구세포 생검 후 배아의 발달도 동결-융해 과정의 영향을 받지 않는 것으로 생각된다. 그러나 착상전 유전진단의 임신율은 동결-융해된 배아에서 낮은 경향을 보였다. 따라서 잉여배아가 있는 경우나 저반응군에서 배아의 수집을 위해서 배아를 동결한 후 융해하여 착상전 유전진단을 유용하게 적용할 수 있으며, 향후 할구세포 생검 전후에 적용할 수 있는 보다 적정한 동결-융해조건을 확립하는 것이 필요할 것으로 사료된다.

P-23 Endothelial Differentiation from Mouse Embryonic Stem Cells

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Background & Objectives: Embryonic stem (ES) cells, first derived from the inner cell mass of mouse blastocysts. Pluripotent ES cells may represent a suitable in vitro model to study vascular development which is difficult to study in experimental animal systems. Recent studies of mutant mice specified a set of molecules involved in vascular development. Among these molecules, VEGF, Flk-1, and VE-cadherin were proven to be requisite for vasculogenesis. Several in vitro systems have been developed for investigating the cellular events in vasculogenesis. The most popular systems are embryoid body formation. Although these culture systems enable to investigate vasculogenesis virtually as it occurs in embryos, the existence of many other lineage cells generated in an uncontrollablemanner hinders understanding of the behavior of endothelial cells. Here, we develop to more simple and mass culture system of mouse ES cells to differentiate endothelial cell lineage.

Method: Cell Culture Undifferentiated mouse D3 ES cells were cultured and used for this studies. Briefly, D3 ES cells were co-cultured with mitomycin inactivated MEF cells in media containing DMEM, 15% FBS and 1,000unit/ml of mouse LIF. Endothelial cell differentiation To initiate ES cell differentiation and EBs formation. Cultures were maintained on EBM2, 5% FBS, VEGF, bFGF, IGF-1, EGF, and ascorbic acid without further feeding for up to 11 days, EBs were collected at different days of differentiation.

Tubule Formation on Matrigel The Matrigel was added to each well of a 24 well tissue culture plate and allowed to solidify at 37℃ for at least 30 minutes. Following gelatinizing, 0.2 ml of a cell suspension containing $5\times10^4 \sim 1\times10^5$ D3 ES cells were placed on top of the Matrigel. Immunostaining Cells were fixed, permeabilized, then incubated at room temperature for one hour with primary antibodies, washed, and incubated for 30 minutes in the secondary antibody. After a final wash, cells were observed by fluorescence microscopy. Reverse Transcriptase-Polymerase Chain Reaction Total RNA was extracted from differentiated EBs with TRIZOL. Reverse transcription was performed using an Superscript II. RT product was used to perform PCR with a Taq DNA polymerase. An initial 3 minute 94℃ hotstart was used followed by cycles consisting of 45 seconds denaturation at 94℃, 45 seconds annealing at 55℃ and 45 seconds extension at 72℃. A 10 minute extension was done at 72℃ after the final cycle. 35 cycles were done for HPRT, Oct-4, Tie-2, VE-cadherin, PECAM and AC133.

Results: 1. EGM-2 culture conditions were effectively induced endothelial differentiation in mES cells. And differentiated mES cells were expressed endothelial specific genes, such as PECAM, VE-cadherin, Tie-2 and AC133. 2. Immunofluorescence data demonstrate several endothelial markers during EB differentiation, reaching a maximum at days 5+2~5. Oct-4, which specific transcription factor and key control molecule for undifferentiation was also expressed during EB formation. However, level of expression were sequentially decreased during cultivation. Some endothelial specific markers were expressed in high levels (Flk-1, PECAM, VE-Cadherin) orlower levels (Tie-2, CD34) became notable after EB formation and differentiation.

Conclusions: We established more simple, efficient differentiation methods of endothelial progenitor cells from mouse ES cells using EGM2 media. Thistool can provide of mass differentiation source of endothelial progenitor cells.

P-24 In vitro Differentiation of Human Embryonic Stem Cells into Osteoblast-like Cells

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Background & Objectives: Bone is maintained by a balance between the synthesis of bone matrix by the osteoblasts and degradation by osteoclasts. Recently, osteogenesis-related transcription factors and osteoblast-specific markers has led to a rapid advancement in understanding the process of osteoblast differentiation. However, the early steps of osteoblast differentiation remain to be identified. In this study, we examine the osteoblast differentiation process in human embryonic stem cells. This experiments have been optimized the culture conditions to achieve osteoblast differentiation from human embryonic stem cell, as measured by both the deposition of a mineralized bone matrix, and the expression of osteoblastic marker genes.

Method: 1) Cell culture and differentiation of embryonic stem cells. Human ES cells were grown on