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-

trifuged at 500 g to remove blood and granulosa cell contaminations. After rehydration, we processed for 2-D PAGE, and Coomassie blue and silver stainings prior to the analysis of the differential protein expressions using ImageMaster™ 2D Platinum software.

Results: In this study, numerous protein spots were identified as being differently expressed in follicular fluids in normal and RSA patients. Seven candidate proteins (1 transcription factor, 1 Zinc finger protein, 1 Tro αH, 1 apolipoprotein, 1 apolipoprotein E precursor, and 2 novel proteins) were identified using matrix-assisted laser desorption/ionization-time of flight-mass spectrometry (MALD-TOF-MS) or peptide sequencing (ESI-Q-TOF-MS/MS). Isolation of these proteins may delineate general health during pregnancy and a better understanding of their cellular functions for maintaining normal pregnancy.

Conclusions: The molecular mechanisms for differential protein expressions have to be elucidated for the prognosis of the pregnancy of individuals with higher risk. Using two-dimensional PAGE and mass spectrometry, all proteins showing increased or decreased expression in RSA will be identified. These proteins are able to use as clinical biomarkers in RSA.

P-16 Cellular Localization and Intensity of Id3 mRNA in Ovaries of Adult Cycling Rats

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Background & Objectives: Inhibitor of DNA binding protein (Id) is another category of mammalian helix-loop-helix (HLH) protein. Four members of the Id family, Id1 to Id4, have been identified in mammalian cells. Id proteins act as positive regulators of cell growth and are required for cell cycle progression in cell line. This study was performed to examine the expression pattern of Id 3 mRNA during folliculogenesis in cycling rat ovary induced by PMSG.

Method: Probes for in situ hybridization studies were made by RT-PCR. All PCR products were cloned into pGEM-T Easy Vector (Promega Corp.). Anti-sense and sense cRNA probes were prepared by means of in vitro transcription using Sp6 or T7 RNA polymerase. Hybridization was carried out with the ^{35}S-labeled RNA probe (1×10^7 cpm/ml) in a solution. The hybridization signal was estimated on a scale of 1+ to 4+; +, silver grains sparse, but positive hybridization; ++, silver grains are numerous but do not cover the cell type in question; +++, silver grains are very numerous and begin to merge in some places; ++++, silver grains are very dense and form a near uniform mass above the cell type in question.

Results: The intensity of the signals showed 2+ or 3+ in oocytes of primordial and primary follicle in no-treatment and PMSG treated ovaries, however, there were no signals in dominant or atretic follicle. The signals showed +/- or 1+ in granulose cells in primordial or primary follicles regardless of PMSG treatment, however, the intensity of the signal in secondary and dominant follicles showed 2+ and 4+, respectively. There were no signals in theca-interstitial or externa cells during folliculogenesis. The most interesting