P39 Estrogen enhances neural differentiation of Human mesenchymal-like stem cells derived from amnion membrane *in vitro*

Hee Jin Goo, Ju Ran Kim, Seong Jin Yu, In Kyung Hong, Na Hee Jeong, Hae Kwon Kim[1], Byung-RoK Do[2], Sung Goo Kang+.

Department of Biotechnology & Biomedical Science, Inje University, Gimhae, Korea, [1]Department of Biotechnology, Seoul Women's University, Seoul, Korea, [2]Biotechonolgy research Institute, Hurim BioCell Inc., Seoul, Korea

Objective: Mesenchymal stem cells (MSCs) have a broad differentiation potential in cell therapy. In this study, we investigated the ability of human amnion mesenchymal cells to differentiate into neural cells under appropriate conditions. Furthermore we examined whether estrogen increases neuronal differentiation.

Materials and Methods: MSCs isolated from human amnion membrane (AM-MSCs) were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum. Cultured cells were immunophenotypically characterized. To induce neural differentiation of human amnion mesenchymal cells, dimethyl sulphoxide (DMSO), butylated hydroxyanisole (BHA) were treated. To confirm the neural characteristics, immunocytochemistry stain for β-tubulin III, GFAP and Gal-C were performed. RT-PCR was performed for detecting NeuroD1, GFAP and MBP mRNA. To determine the effect of estrogen on neural differentiation and axonal growth, E_2 and ICI-118,780, estrogen antagonist, was added to the neural induction medium.

Results: AM-MSCs isolated from amnion membrane expressed neuro-glia markers (β tubulin III, GFAP and Gal-c) in appropriate condition. The expression proportion was about 36% for neuron, 14% for astrocyte, 16% for oligodendrocyte. In the presence of estrogen, the proportion of neuron was increased to 43% ($p<0.05$). Increase in neurons was abrogated by an estrogen receptor (ER) antagonist, ICI, about 36%.

Conclusion: AM-MSCs derived from amnion membrane can be differentiated into neuroglial phenotypes by optimal differentiation protocol. Furthermore AM-MSCs treated with estrogen can be increased growth of neurons. These results indicate that AM-derived MSCs may represent an advantageous source of progenitor cells with potential applications in a variety of cell therapy and transplantation procedures and estrogen treatment would be more effective for lesion restoration.

Key words: Amnion mesenchymal stem cell, neuronal differentiation, estrogen, neurite growth

P40 Various hormonal conditions that affect quality of immature oocytes in PCOS patients

Chung MK, Park EA, Lee DR, Cho JH, Sohn JO, Han SY, Yoon TK, Cha KY

Fertility Center of CHA General Hospital, CHA Research Institute, Pochon CHA University, Seoul, Korea

Objectives: Retrieval of immature oocytes followed by *in vitro* maturation (IVM) of these oocytes has an important value in infertility treatment. This technique can rescue immature oocytes, control the treatment for patients having various endocrine characteristics and overcome the limitation of conventional assisted reproductive technology (ART) systems. IVM-IVF combined with an unstimulation program in PCOS patients, which has a high risk of ovarian hyperstimulation syndrome could be one of the most effective procedures in human ART. Thus far, the success rate of *in vitro* maturation program is still low. For improving the IVM-IVF-ET outcome, we analyzed the various conditions that affect quality of immature oocytes in PCOS patients between the hCG primed and non primed cycles.

Materials and Methods: Retrospective and compared study of IVM-IVF-ET cycles between hCG primed and non primed before oocyte retrieval; In IVM-IVF-ET cycles of hCG primed (n=36, 33 patients; 30.8 ± 2.9 yrs) and non primed (n=184, 138 patients; 31.2 ± 3.2 yrs) cycles, IVF outcomes (number of retrieved, cultured, matured, fertilized, cleaved oocytes, and maturation and fertilization rates) correlated with oocyte quality were analyzed according to the patient's age, basal hormonal levels (FSH, LH, E_2 and Prolactin) and LH/FSH ratio. Statistical analyses were performed using Pearson correlation, Student's t-test or Chi-square test.

Results: Between the hCG primed and non primed cycles, no. of collected and matured oocytes presented statistical significance (p value; 0.037, 0.038), but no. of fertilized, cleaved oocytes, maturation rates and fertilization rates presented no statistical differences. In hCG non primed cycles, number of retrieved and cultured oocytes were significantly higher in >34 of patient age (p=0.002, 0.001) group and maturation rates were significantly higher in <35 of patient age (p=0.002) group. Unlike hCG non primed cycles, basal level of FSH had effect on the number of retrieved, cultured and matured oocytes (p=0.020, 0.018 and 0.049). Significantly higher fertilization rates were shown according to elevated LH/FSH ratio (p=0.007). Also, in hCG primed cycles, concentration of prolactin had effect on the no. of collected/ cultured/ matured/ fertilized and cleaved oocytes (p=0.048, 0.033, 0.003, 0.027, 0.049).

Conclusions: This study demonstrates that priming of hCG may increase the number of retrieved immature oocytes than the retrieval of good quality oocytes. However, the higher level of basal FSH and the lower level of basal prolactin were correlated with the retrieved number of good quality oocytes in hCG primed group. And, the higher level of LH/FSH ratio was correlated with the oocytes quality after retrieved and cultured. Based on these results, new patient selection criteria are required to retrieve numerous number of good quality oocytes. And improved *in vitro* culture system should be developed to elevate the clinical results for IVM.

Support: This work was supported by a grant from the specialized program of the University funded by the Ministry of Education and Human Resources Development, Republic of Korea.

Key words: PCOS, hCG, IVM, Hormonal level, Outcome