P-34 Mechanical Culture of Human Endometrial Cells (EEC and HEC-1B) and Mouse Embryos

JB Lee[1,2], HC Lee[1], YS Cho[1], JK Won[1], YJ Cho[1], KH Lee[1], SY Kim[1], JJ Lim[1], SK Kim[1], HJ Lee[1], BH Min[2], CJ Chung[1]

[1]Infertility Center, Shin Women's Hospital, Gyonggi, Korea
[2]Department of Pharmacology, College of Medicine, Korea University, Seoul, Korea

Objective: To investigate the effect of mechanical culture system on the growth of human endometrial cell as well as development of mouse embryos.

Materials and Methods: Endometrial samples for primary culture of human endometrial epithelial cell were obtained from human hysterectomy specimens and biopsies, and HEC-1B cell line was obtained from American type cell collection (ATCC). Endometrial-epithelial cells (EEC and HEC-1B) were cultured with initial concentration of 1×10^5/ml to 96 hours and counted at 24 hours interval for proliferation rate. For observation of embyonic development and count of total cell number of blastocyst, embryos were collected from mated mice of superovulation with 5 IU PMSG and 5 IU hCG. Total cell count of blastomere was performed under fluorescent microscope after Hoechst staining. We utilized a shaking machine (mylab-shaker SL30, Korea) in a humidified atmosphere of 5% CO_2 and 95% air at 37°C for mechanical culture system.

Results: The growth rate of human endometrial epithelial cells both EEC and HEC-1B was slightly decreased in the mechanical culture system compared to general culture system. However, In the development of mouse embryo, both hatching blastocyst rate and total cell number of mouse blastocysts on day 6 were significantly increased in mechanical culture compared to control group (75.7% vs. 44.4%: 77.9± 3.7 vs. 63.2±4.0; $p<0.05$). The blastocyt rate and the total cell number of mouse blastocysto (on day 5) in mechanical group were not different from that in control (83.8% vs. 70.9% ; 92.2±3.3 vs. 80.7±4.7).

Conclusion: Mechanical culture does not have an effect on the cell growth rate of human endometrial epithelial cell, however it increase hatching blastocyst rate and total cell number of blastocyst in mice. It seems to enhance the development of mouse embyos *in vitro* and provide a more physiological environment by using shaking-culture *in vitro* instead of mobile environment in reproductive tract.

P-35 The Effects of Glucose on Blastulation and Cell Counts of Blastocysts in Mice

SB Park[1], JC Kim[1], KS Park[2], TH Lee[2], SS Chun[2], HB Song[1]

[1]Division of Life Resources, Daegu University, Gyungbuk, [2]Department of OB/Gyn, Kyungpook National University Hospital, Daegu, Korea

Objective: The aim of this study was to investigate the effect of glucose on embryonic development of

mouse embryos.

Materials and Methods: Two cell embryos were recovered from ICR female mice (3~4 weeks) at 46~50 hrs after hCG 5 IU injection (mated just after hCG injection) and cultured in 50 μm DMEM droplets supplemented with nothing (control: n=46), glucose 0.5mM (Group A; n=46) or glucose 3.15 mM (Group B; n=46) under mineral oil. All experimental media were supplemented with 20% human follicular fluid.

Results: Total blastocyst formation rates was lower (NS) in glucose groups (group A: 52.2%; B: 47.8%) than control group (60.9%). ZiB rates was the highest ($p<0.05$) in control (47.8%) than those in group A (21.7%) and B (28.3%). ZeB rates were the highest (NS) in group A (30.4%) than those in control (13.0%) and group B (19.6%). Blastocysts, cultured in group B (50.5), had the highest (NS) mean cell number compared with the others (control: 39.2; group A: (45.6). The ICM proportion (%ICM of total cells) in blastocysts cultured in group A (20.6%) was the highest (NS) than those of other tested groups (control: 15.2; group B: 13.9%).

Conclusion: This study shows that a low dose (0.5 mM) of glucose added to culture medium increases the developmental capacity of 2 cell embryos in mice.

Key Words: Mouse 2 cell embryo, Glucose, Blastulation, Cell number, ICM proportion

P-36 Isolation of Novel Deubiquitinating Enzymes in Human Chorionic Villi

JM Shin, SK Lim[2], KJ Yoo[1], SH Lee[2], KH Baek[1,2]

Graduate School of Life Science and Biotechnology[1], College of Medicine, Pochon CHA University, Infertility Medical Center[2]

Background & Objectives: To isolate and characterize of novel human deubiquitinating enzymes in human chorionic villi.

Method: To identify novel deubiquitinating enzymes in human chorionic villi, we generated five degenerate PCR primers based on the conserved sequences for catalytic domains (Cys, two Asp and two His) of deubiquitinating enzymes. Expression pattern were confirmed by RT-PCR and Northern blotting. Ub-b-gal assay was performed in E. coli to confirm in vitro functional assay.

Results: We obtained multiple bands from various cell lines and tissues by RT-PCR using degenerate PCR primers. Sequence analysis revealed that some of unknown genes contained conserved domains which are necessary for deubiquitinating activity and high homology with putative human DUBs. By using NCBI BLAST algorithm information, we isolated a novel human DUB enzyme in chorionic villi, and named vDUB3. The full-length vDUB3 cDNA has 1,593 bp and encodes a 530 amino acid polypeptide with the molecular weight of approximately 58 kDa. This enzyme also contains the highly conserved catalytic domains and biochemical assay revealed that vDUB3 has deubiquitinating activity in vitro.

Conclusions: Taken all together, we propose that vDUB3 is a novel human DUB enzyme. And, we con-