ng와 연속적으로 희석한 competitor를 같이 competitive PCR하였다.

Results: 미세정자주입술로 임신한 그룹과 정상 임신 그룹 어머니의 평균 나이는 각각 33.94세와 32.49세 였다. nested PCR을 통한 common deletion의 분석결과는 미세정자주입술 그룹에서 81.1%, 정상 그룹에서 33.3%였다. Competitive PCR로 나타난 common deletion의 정량분석에서는 미세정자주입술 그룹과 정상 그룹이 각각 0.002405, 0.002171로 차이가 없었다.

Conclusions: 결과에 따르면, 미세정자주입술 그룹이 정상 그룹보다 common deletion의 비율은 높았으나, 실질적인 양을 비교해 보면 차이가 없었다. 포유동물에서 미토콘드리아는 수정시 난자로부터 유전된다고 알려져 있고 그 유전현상은 여전히 연구대상이다. 그러므로 수정란 내 세포질이 정자 미토콘드리아를 인식하고 없어지는 종 특이적인 기작이 존재하는지에 대한 연구가 필요하다.

본 연구는 보건복지부 보건의료기술진흥사업의 지원에 의하여 이루어진 것임.
과제고유번호: 01-PJ10-PG6-01GN13-0002.

O-17 Differential Expression of id-1in ovx/estrogen Treatment Mouse Uterus

HY Nah, SH Hong[1,2], JY Lee[1], YJ Lee[1,2], SH Kim[1], HD Chae[1], CH Kim[1], BM Kang[1]

Department of Obstetrics and Gynecology, College of Medicine, Ulsan University, Asan Medical Center, Seoul[1], Department of Life Science, College of Natural Sciences, Hanyang University, Seoul, Korea[2]

Background & Objectives: The helix-loop-helix (HLH) protein class of transcription factors is important regulators of cellular proliferation and differentiation in a number of cell types. Recently, Chaudhary and colleagues have reported that Id genes may be involved in the differentiation and hormonal regulation of Sertoli cell. In this study, we examined the differential expression of Id-1 protein and gene in OVX/estrogen treatment/6-h/12-h protocols.

Method: Female ICR mice (6~7 weeks old) were ovariectomized and rested for 14 days before receiving estrogen treatment. They were injected S.C with oil and 17β-estradiol which was dissolved in sesame oil. Mice were killed and uterine horns were collected at 6 h or 12 h after injection. Adult female mice were placed with fertile males of the same strain, and the day that a vaginal plug was found was considered as day 1 of pregnancy. On the evening, of day 4 at the time of blastocyst attachment, implantation sites were visualized by intravenous injection of Chicago Blue B solution before killing the mice. Implantation segments containing implanting embryo were finely separated from nonimplantation segments. After we performed cDNA microarray in the OVX/estrogen treatment/6-h/12-h protocols, confirmed the expression patterns of Id-1 by RT-PCR and Laser Capture Microdissection (LCM). We localized Id-1 protein by immunohistochemistry (IHC).

Results: We performed cDNA microarray to search estrogen-responsiveness genes in the mouse uterus and so shown that Id genes were regulated by estrogen in the OVX/estrogen treatment/6-h/12-h protocols.

Id-1 gene was higher expressed than other genes in the OVX/estrogen treatment/6-h/12-h protocols among Id genes. To investigate the differential expression pattern of Id-1 gene, we performed LCM and then identified that Id-1 mRNA was only expressed in the epithelium of the OVX/estrogen treatment/6-h/12-h protocols. Also, Id-1 protein was predominantly localized in luminal and glandular epithelium of the OVX/estrogen treatment/6-h/12-h protocols. Interestingly, the expression of Id-1 mRNA was shown to upregulate at the implantation sites compared to interimplantation sites.

Conclusions: These results revealed that Id-1 was strongly regulated by estrogen in the mouse uterus. Particularly, we suggest that Id-1 may be possessed unidentified function in epithelium of the mouse uterus. The observations presented in the current study suggest that Id-1 gene may have a distinct function in the uterine physiological events, such as the implantation process and the estrus cycle. However, we do not know the molecular mechanisms which regulate the expression of Id-1 genes between the uterus and blastocysts and how steroid hormone might regulate the expression of Id-1 is not clear.

O-18 Ceramidase Inhibition Potentiates Stress-induced Apoptosis of Murine Ovarian Granulosa Cells

MR Kim, JH Kim[1], HH Jo[1], DJ Kwon[1], YT Lim[1], J.L. Tilly[2], JH Kim[1]

Department of OB/GYN, The Catholic University of Korea, [1]Vincent Center for Reproductive Biology, Department of OB/GYN, Harvard Medical School[2]

Background & Objectives: (1) To evaluate the effect of ceramide versus ceramine in regulating murine granulosa cell death (2) To examine the mRNA expression of ceramidase in granulosa cells and the effect of inhibition of ceramidase in stress-induced granulosa cell death

Method: In Vivo: Ovaries were collected from C57BL/6 (22~24 days postpartum) female mice prior to and 42 hours after in vivo stimulation with gonadotropin hormone (PMSG; 5 IU) to promote follicle and granulosa cell survival. In addition, corpora lutea were collected from gonadotropin-stimulated (PMSG + hCG) ovaries 84 hours after the start of treatment (approximately 30 hours after ovulation). Northern blot hybridization and in situ hybridization were used to evaluate mRNA expression of acid ceramidase. In Vitro: Female C57BL/6 mice (22~24 days postpartum) were treated with PMSG (5 IU, n=3). Ovaries were collected 42 hours later and healthy antral follicles were punctured to extrude granulosa cells. Granulosa cells were pre-cultured in Waymouth medium with 10% fetal bovine serum for 24 hours. Media from all cultures were replaced with media lacking serum and cultures were continued for 6~24 hours with ceramidase inhibitors; oleylethanolamide (OE) or (1S, 2R)-D-erythro-2-(N-myristoylamino)-1-phenyl-1-propanol (D-MAPP) with ceramide or serum starvation. Cells were fixed with 4% paraformaldehyde and stained with Hoescht for examination of nuclear morphology (apoptosis). Diacylglycerol kinase assay was used to measure ceramide levels.

Results: After 6 hours, C8-ceramide (50 mM) triggered apoptosis in only 28 6% of the cells, whereas C8-ceramine (50 mM) induced apoptosis in all cells (LD_{50} = 1 mM). These data, which suggest that cera-