Method: 1 μM 또는 10 μM SNP가 함유된 5 I.U. PMSG 0.1 cc를 1) 6~9주령 (I군), 2) 14~16주령 (II군), 20~26주령 (III군)의 크게 3종류의 연령대의 자성 생쥐 (C57BL)에 복강 내 주사하였다. SNP가 함유되지 않은 5 I.U. PMSG만을 동일 연령의 생쥐와 6주령의 생쥐에 주사하여 각각 음성 대조군과 양성 대조군으로 이용하였다. PMSG 주사 48시간째 0.1 cc의 5 I.U. hCG를 복강 내 주사하고, 즉시 10~12주인 가임 능력이 확인된 웅성 생쥐와 1:1 합사시켰다. 질전이 관찰된 생쥐에서 hCG 주사 후 18시간째 나팔관의 팽대부로부터 배아 회수가 이루어졌다. 자성 생쥐 1마리당 채취된 배아 수, 채취된 전체 배아 가운데 단편화가 있는 비정상적 형태의 배아의 비율, 및 포배아 형성율을 관찰하였다. 배아 채취 후 각 군의 난소 조직은 제거하여 Western blot 및 면역조직화학염색 방법으로 VEGF 발현을 조사하였다.

Results: SNP와 PMSG의 동시 투여가 6~9주령 및 14~16주령의 자성생쥐에서는 채취된 배아 수와 포배아 발생률에 대해 PMSG만을 투여한 대조군과 차이가 없었다. 그러나 보다 주령이 높은 20~26주령의 자성생쥐에 SNP와 PMSG를 투여했을 경우 채취한 배아 수와 포배아까지의 발생률이 대조군에 비해 유의하게 증가하였으며, 특히 대조군에서의 포배아 발달률은 1.5%로 대부분이 2- 또는 4-세포기 단계에서 배 발달이 억제된 반면 SNP를 투여한 경우 SNP 농도에 의존적으로 포배아 발달률이 증가하였다 (1 μM SNP: 35.6%, 10 μM SNP: 47.4%). Western blot 방법으로 VEGF 발현 양상을 조사한 결과 SNP 투여 효과가 없었던 14~16주령의 난소에서는 VEGF의 발현이 SNP 투여 농도에 관계없이 대조군과 차이가 없었으며, 오히려 농도 의존적으로 다소 감소하는 경향을 보였다. 그러나 SNP 투여 효과가 있었던 20~26주령의 고령 자성생쥐 난소에는 SNP 농도에 의존적으로 VEGF의 발현이 대조군에 비해 유의하게 증가하였고 면역조직화학염색 결과 주로 과립막세포에서 관찰되었다.

Conclusions: 고령 생쥐의 과배란유도시 성선호르몬과 함께 SNP의 동시 투여가 어린 생쥐와 비슷한 배아 수와 배아 발달 능력을 지닌 난자를 유도하며, 이러한 결과가 SNP로부터 생성된 NO에 의한 혈관생성 촉진인자인 VEGF의 난소 내 발현 증가와 관계가 있는 것으로 생각된다.

P-3 Association Study for Single Nucleotide Polymorphisms in INSR Gene and Polycystic Ovary Syndrome

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Background & Objectives: Polycystic ovary syndrome (PCOS) is an endocrine disorder which is frequently shown in premenopausal women and a number of patients with PCOS have symptom of high risk for type 2 diabetes.

Method: To evaluate associations between several single nucleotide polymorphisms (SNPs) of the insulin receptor (INSR) gene and PCOS in a Korean population, we have sequenced all exons of INSR to discover SNPs. After selecting 9 candidate SNPs which include +109482 A>G, +109665 C>T, +125498 A>G, +127527 G>A, +143485 G>C, +161822 G>A, +168606 C>T, +168828 T>A and +176477 C>T, we recruited 134 women with PCOS and 100 healthy women. For genotyping of polymorphic sites, we used

TaqMan SNP genotyping assays. Also, we used HapAnalyzer for association studies.

Results: 8 analyzed SNPs of INSR in this report including +109482 A>G, +109665 C>T, +125498 A>G, +127527 G>A, +143485 G>C, +161822 G>A, +168606 C>T and +168828 T>A are not associated with PCOS in a Korean population due to the fact that they had similar frequencies of three genotypes between PCOS and control groups. And the frequency of minor allele for +176477 C>T in INSR was significantly higher in control group than in the patient group.

Conclusions: In this study, we identified a novel SNP in INSR gene (+176477 C>T), which shows the significant association with PCOS. The frequency of this minor allele was much higher in a control group than in the PCOS patient group at a significant level (p=0.0401). From this result, we suggest that the minor allele T in +176477 C>T of INSR gene may have a protective effect in pathogenesis of PCOS in a Korean population.

P-4 Effects of Media on Blastocyst Quality and Pregnancy Rate in Mouse and Human

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Background & Objectives: The aim of this study was to investigate the effects of two different media on blastulation, blastocyst quality and pregnancy rate in both mouse and human.

Method: In mouse, total of 376 two cell embryos were retrieved from ICR female mice (3~4 weeks old) at 48 h after 5 IU hCG injection (mated just after hCG injection) and cultured in TCM (n=138) and MEM (n=138) respectively. The blastocysts were graded from zona-intact (ZiB) to zona-escape (hatching and hatched, ZeB) at 72 h after culture. Total TE and ICM cell numbers of blastocysts were analyzed after differential staining. In human, total of 49 couples (TCM or MEM in sibling: n=10; TCM: n=20; MEM: n=19) were included in this study. Developmental capacity of oocytes was evaluated with the sibling oocytes of same patients cultured in TCM or MEM. Clinical pregnancy rate was evaluated with the transferred blastocysts of different patients cultured in TCM or MEM. Blastocysts were graded (BG1, BG2, BG3 and early) on day 5~7, and transferred (n=2~4) on day 5. Statistical analysis was performed using χ^2 and Student's t-test and considered statistically significant when p-value was < 0.05.

Results: In mouse, blastulation rate (BR) and ZiB rate in MEM (66.7% and 33.3%) were significantly higher ($p<0.05$) than those in TCM (59.4% and 25.4%). No difference was found in ZeB rate between MEM and TCM (32.6% and 34.1%). Total of 160 blastocysts (TCM: n=79; MEM: n=81) were stained. Mean cell number of blastocysts was significantly higher ($p<0.01$) in TCM (70.0) than that in MEM (57.8). Differential staining was successfully performed in 70 blastocysts (TCM: n=37; MEM: n=33). The percentage of ICM was significantly higher ($p<0.05$) in MEM than that in TCM (20.6% vs. 16.9%). However, the ICM: TE ratio was significantly higher ($p<0.05$) in TCM than that in MEM (1:4.92 vs. 1:3.86). In human, there were no statistically significant differences in the rate of fertilization, cleavage and total