보고, 배반포에서 배발달과 착상에 관련 유전자로 알려진 IGF-1, IGF-II 그리고 LIF의 발현양상을 조사하고자 수행하였다. 그리고 이러한 결과를 임상에 적용하여 임신율에 대한 향상 효과를 살펴보았다.

대상 및 방법: 기본배양액으로는 P-1 배양액을 이용하였으며, 2-세포기 수정란은 6~8주령의 ICR 계통의 생쥐로부터 hCG 주사 후 46~48시간째 회수하였으며, 회수된 수정란은 0, 1, 5 그리고 10 ng/ml 농도의 GM-CSF가 첨가된 배양액 소적에서 배양을 실시하였다. 배반포의 세포수를 측정하기 위하여 hCG 주사 112시간째에 고정시킨 후 Hoechst 33342로 염색하여 형광현미경하에서 관찰하였다. 배발달 관련 유전자인 IGF-1, IGF-II 그리고 착상 관련 유전자인 LIF의 발현양상을 배반포에서 확인하기 위해서 RT-PCR을 이용하였으며, band intensity는 densitometry를 이용하여 발현정도를 비교 분석하였다. 인간 시험관아기 프로그램에 있어서는, 과배란유도에 의하여 난자를 채취하였으며, 정상적인 체외수정과 ICSI에 의하여 수정을 유도한 후, 난자채취 3일째 자궁강 내에 이식을 실시하였다.

결 과: 배양액내에 GM-CSF의 첨가에 따른 배반포의 발달율과 부화율은 1 ng/ml (68.6, 36.4%), 5 ng/ml (73.0, 43.2%) 그리고 10 ng/ml (76.1, 53.0%) 첨가군이 대조군 (65.5, 35.2%)에 비하여 농도의존적으로 높은 경향을 나타냈으며, 특히, 10 ng/ml 첨가군에서 가장 높은 발달율과 부화율을 나타냈다. 이러한 결과를 기초로 이후부터는 10 ng/ml GM-CSF 첨가군과 대조군을 비교 실험하였다. 먼저 hCG 주사 112시간째 배반포의 세포수는 GM-CSF 첨가군 (52.0±9.4)과 대조군 (49.8±11.1)간에 차이가 없는 것으로 나타났다. 그렇지만, hCG 주사 168시간째 완전히 부화된 배반포의 비율은 GM-CSF 첨가군 (75.7%)이 대조군 (23.5%)에 비하여 유의하게 높은 것으로 조사되었다. 배반포에서 유전자의 발현정도를 비교한 결과는 배발달에 중요한 역할을 하는 IGF-1, IGF-II 유전자의 발현은 두 군간에 차이를 나타내지 않았지만, 착상과정에서 중요한 역할을 하는 것으로 알려진 LIF 유전자의 발현은 GM-CSF 첨가군이 대조군보다 유의하게 높은 발현양상을 보여주었다. 한편, GM-CSF를 임상에 적용한 결과, 임상적 임신율이 47.4% (45/95)로서 대조군의 33.3% (32/96) 보다 유의하게 향상되는 것으로 나타났다.

결 론: 배양액내에 GM-CSF의 첨가는 생쥐 수정란의 발달을 증가시키고, 특히 부화율을 증가시키며, 또한 착상 관련 유전자 LIF의 발현을 유의하게 증가시킴으로서 체외배양된 수정란의 발생능력을 향상시키는 것으로 사료된다. 또한 GM-CSF의 임상적 이용은 인간 수정란의 발생능력을 향상시킴으로서 보다 높은 임신율 얻기 위한 배양액 첨가제로서 유용하게 이용될 수 있으리라 사료된다.

B-13 Chromatin and Microtubule Organization in Maturing and Preactivated Oocytes Following Sperm Injection

Kim BK, Lee YJ, Cui XS and Kim NH

Department of Animal Science, Chungbuk National University, Cheong Ju, Chungbuk, Korea

Objective: Chromatin and microtubule organization were determined in porcine maturing and activated oocyte following intracytoplasmic sperm injection in order to obtain insights into the nature of decondensation ability of sperm nucleus and nucleation activity of microtubules in mammalian oocytes.

Method: An porcine oocyte was penetrated by the injecting mocropipette, a small amount of cytoplasm was drawn into the micropipette, and then the cytoplasm together with the sperm and a small amount of medium was expelled into the oocyte. Microtubule localization was confirmed using a mouse monoclonal antibody to α-tubulin and detected using a fluorescent labeled goat anti-mouse secondary antibody. DNA

was stained with propidium iodide. The image of microtubules and chromatin was captured using laser scanning confocal microscope.

Result: In germinal vesicle stage oocyte, sperm chromatin remained condensation and sperm derived microtubules were not observed at 8 to 10 h after sperm injection. At 18~20 h after injection, the sperm nucleus developed to the metaphase chromatin along the metaphase structure of female nucleus. In some metaphase I stage oocytes, sperm chromatin decondensed at 8 h to 10 h after injection, sperm aster was seen soon after sperm injection. At 18~20 h after sperm injection into metaphase I stage oocyte, male chromatin developed to the metaphase chromatin while female chromatin extruded first polar body and formed the metaphase chromatin. At 18 to 20 h after sperm injection into preactivated oocytes, condensed sperm nucleus was located in close proximity of female pronucleus. However, the condensed nucleus did not fuse with female pronucleus. In preactivated ocytes, injected sperm remained condensation, a few sperm organized small microtubular aster. Instead, maternal derived microtubules were organized near the female chromatin, which seem to move condensed male chromatin near to the female pronucleus.

Conclusion: Sperm nuclear decondensing activity and microtubule organization activity of centrosome during fertilization are cell cycle dependent. In absence of male functional centrosome, female origin centrosome takes over the role of microtubule nucleation for nuclear movement.

B-14 Effect of Spermatogenesis by L-type and T-type Ca^{2+} Channel Blocker in Mice

Lee JH[1,3], Lee SJ[2], Kim H[3], Kim NY[1] and Lee SJ[1]

[1]Laboratory of IVF, Mirae and Heemang Ob/Gyn Clinic, Kangnam-Gu, [2]Department of Animal Science, Sahmyook College, Nowon-Gu, [3]Department of Biotechnology, Seoul Women's University, Nowon-Gu, Seoul, Korea

Introduction: Spermatogenesis is a complex and highly coordinated process by which spermatogonia proliferate and differentiate to produce mature sperm. There are ample evidences indicating that elevations of intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) are key signals for spermatogenesis, although little is known about its regulation in spermatogenic cells. A functional low voltage-activated Ca channel is expressed during the meiotic and post-meiotic stages of mammalian spermatogenesis. Pharmacological and physical analyses suggest that a Ca channel operation is required for actual spermatogenesis. This study was conducted to investigate the effect of L-type or T-type calcium channel blocker on in vivo spermatogenesis in mice.

Materials and Methods: ICR male mice of 18 day old were used. Nifedipine, a L-type Ca^{2+} channel blocker, was administered orally for 20 days at dosages of 20, 40 or 80 mg/kg body weight. Ethosuximide (Sigma, USA), a T-type Ca^{2+} channel blocker, was administered at 100, 500 or 1000 mg/kg body weight. Dosages were within the range of the general physiological treatment. The control group was fed with distilled water. After 20 days, spermatogenesis was investigated by measuring the size of testis and number of sperm in seminiferous tubules. The slides were stained with H/E staining following the standard fashion for pathological evaluation. Serum concentrations of LH, prolactin and testosterone were analyzed by radio-