Integrin $\alpha v \beta 3$ (vitronectin receptor, VTN)은 정상적인 생리주기를 갖는 여성에 있어서 implantation window (day20-24)에 발현되는 중요한 integrin으로 알려져 있다. 본 실험은 IVF 환자에 있어서의 implantatin window의 변화를 VTN을 이용하여 관찰하고자 하였다. 실험에 공여된 자궁내막조직은 난자 회수 2일 후, 즉 배아 이식(embryo transfer) 당일, 수정 실패로 인해 배아 이식이 불가능한 환자 11명을 대상으로 조직생검하여 획득하였다. 11명중 7명은 FSH/hMG/hCG 로 과배란을 유기한 IVF 환자였고, GnRHa/FSH/hMG/hCG를 사용한 IVF 환자가 4명이었다. 획득한 조직은 생리식염수로 세척한 후, 10% 포르말린 용액에서 고정하였고 파라핀 절편으로 제작되었다. 황체기 결정은 보고된 standard criteria에 따라 자궁내막 조직을 dating하였으며, VTN의 면역조직화학적 분석은 파라핀 절편으로 제작된 조직에 상용되는 항체를 사용하였다. Vitronectin receptor의 positive control로는 생리주기 23-24일인 여성의 자궁내막 조직을 이용하였고, negative control로는 1차 항체 대신 1% BSA를 사용하여 실험하였다. 자궁내막의 이차원 구조는 헤마톡실린과 에오진 염색방법으로, vitronectin receptor의 발현은 면역조직 화학적 방법으로 확인하였다. 그 결과, 11명의 IVF 환자에서 획득한 자궁내막조직 모두에서 VTN의 발현이 확인되었으며, 선상피나 자궁내막 상피조직에 비해 기질 (stroma)에서 좀 더 많이 발현됨을 관찰하였다. 또한 H-E염색을 통한 dating에서는 IVF 환자의 자궁내막 조직이 정상조직의 발달정도에 비해 큰 차이는 없었으나 edema의 분포와 분비선 (gland)의 형태가 불안정함을 알 수 있었다. 결론적으로, IVF 환자에서의 VTN의 발현이 배아 이식 당일에 이미 발현되어 있음을 알 수 있었으며, 따라서 IVF 환자에서 implantation window 시기의 변화가 있는 것으로 사료된다.

P-29

Effect of Ethanol Stimulation Before *In Vitro* Fertilization on Polyspermy and Subsequent Early Development in Porcine Oocytes

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Following gamate fusion or artificial activation, cortical granule (CG) fuse with the overlying oolemma and extrude their contents into the perivitelline space (PVS) in an exocytotic event termed the cortical reaction. CG contents released into the PVS are thought to be responsible for establishing a block to polyspermy at the ZP and may function in an oolemma and/or PVS block. The problems for porcine oocytes are polyspermy following *in vitro* maturation (IVM) and *in vitro* fertilization (IVF). It has been reported that a reason of polyspermy is due to the exocytosis of CG occurred slowly and incompletely during IVF. In the present study, we examined the effects of ethanol (EtOH) stimulation before IVF on polyspermy and subsequent early development. Porcine oocyte-cumulus cell complexes from ovaries were cultured in NCSU23 medium supplemented with 0.6 mM cysteine, 2 $\mu g/m\ell$ FSH, and 10% porcine follicular fluid for 42 h of maturation. After maturation in culture, oocytes were co-cultured with spermatozoa in Tris-buffered medium supplemented with 5 mM caffeine and 0.4 % BSA. Oocytes were divided into three groups. 1) Oocytes were inseminated following EtOH (10%) stimulation for 3 min. 2) Oocytes were inseminated commonly. 3) Oocytes were stimulated with EtOH (10%) only. At 12 h after insemination, some oocytes were fixed for examining sperm penetration and formation of pronuclei. Another oocytes was further cultured in NCSU23 medium supplemented with 0.4 % BSA for 7days. The results indicated that although EtOH stimulation before IVF did not significantly decrease the percentage of polyspermy, there was a tendency to decrease the

mean numbers of penetrated sperm in each oocytes. There were no significant differences in the early development of oocytes between EtOH stimulation before IVF of oocytes and common IVF oocytes. These results suggest that the exposure of porcine oocytes to an EtOH stimulation before common IVF might be associated with late sperm penetration to induce cortical reaction completely, which might reduce the mean number of penetrated spermatozoa in each oocytes.

P-30

Comparative Aspects of Immature Human Oocytes Derived from Stimulated and Unstimulated Ovaries

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It is not clear how is the time course of GVBD and maturation when the GV-stage oocytes derived from stimulated and unstimulated ovaries. It is also nuclear how is different in fertilization and early development between the immature human oocytes derived from stimulated and unstimulated cycles. The purpose of the present study was to compare the time course of oocyte maturation and the capacity of fertilization and cleavage with the immature human oocytes retrieved from stimulated and unstimulated ovaries. Immature GV-stage oocytes were obtained from 35 women in IVF-ET programs. The stimulation protocol for these patients was GnRH agonist with FSH/hMG. Immature GV-stage oocytes were collected from unstimulated ovaries of 12 consented donors. Oocytes were matured in TCM-199 supplemented 20% fetal bovine serum, 10 IU/ml PMSG, 10 IU/ml hCG and 0.2 mM pyruvate. One to three oocytes were cultured in 2 ml of maturation medium in the two well organ culture dish at 37 ℃ in an atmosphere of 5% CO_2 in air. For time course of oocyte GVBD and nuclear maturation, oocytes were observed 3 h interval under dissecting microscopy in a warm (37℃ 5% CO_2) chamber. For insemination, a male factor caused infertility was excluded from this experiment. GV-stage oocytes from stimulated ovaries were inseminated with fresh husband semen. GV-stage oocytes from unstimulated ovaries were inseminated with donor fresh semen. Fertilization medium was TCM-199+20% follicualr fluid (FF). Oocytes that had been fertilized were identified when two pronuclei were present in the cytoplasm. Following observation, the oocytes were transferred into TCM-199+20% FF for further developmental culture. At 48 h after insemina- tion, the oocytes were observed for cleavage. The results of the present study indicated that the time course of GVBD and maturation were different between the oocytes retrieved from stimulated and unstimulated ovaries. Most of oocytes matured to M-II stage were at 30 h after culture in vitro in the oocytes derived from stimulated ovaries and were at 45 h after culture in vitro in the oocytes derived from unstimulated ovaries. However, there was no significant difference in maturation rate between the two groups (75.0% vs 77.5%). The fertilization rate were significant difference between the oocytes retrieved from stimulated and unstimulated ovaries respectively (54.6% vs 92.6%; $P<0.01$). However, the cleavage rates were not different between the two groups (83.3% vs 88.0%). These results suggest that low fertilization rate with stimulated oocytes may be due to the altered characteristics of the zona pellucida.

P-31

한국인 스와이어 증후군 환자 및 가계 구성원의 SRY 유전자 변이의 양상