

으로 이동, 개체로 발생하게 되나 수정이 되지 않을 경우 퇴화한다. 또한 난자와 함께 배란된 난구세포는 난자의 수정여부와 상관없이 반드시 퇴화하여 제거되는데, 그 기작에 대해서는 구체적으로 알려져 있지 않다. 본 연구에서는 소의 수란관 조직추출액이 생쥐의 난구세포와 난자의 생존에 미치는 영향을 조사하였다.

대상 및 방법: 생후 8주된 생쥐에 PMSG 및 hCG를 주사하고 15~16시간 후에 수란관으로부터 난자-난구의 복합체를 얻어 enzyme 처리 후 난구세포를 배양하였다. 배양된 난구세포에 수란관 조직추출액을 10% 처리한 후 48시간에 세포의 형태를 관찰하고, DAPI, PI 염색을 하여 핵의 상태를 확인하였다. 이러한 효과를 나타내는 물질이 무엇인지 알아보기 위해 수란관 조직 추출액을 열처리 (65℃, 90℃)하거나 분자량의 크기 (100 kDa)를 나누어 조사해 보았다. 또한 수란관 조직 추출액으로부터 지방을 추출하여 처리하고 Oil Red O를 이용하여 염색한 후 관찰하였다. 난자의 체외배양시 open culture method와 oil-drop culture method를 이용하여 수란관 조직 추출액의 영향을 비교, 조사하였다.

결 과: 난구세포의 배양시 수란관 조직 추출액을 처리한 경우, 세포의 형태가 변화하며 결국 죽어가는 양상을 보였다. 열처리 후 처리한 경우는 그 효과가 더욱 증가되는 것으로 나타났으며, 분자량의 크기가 100 kDa 이상인 분획에서 더 많은 세포가 죽는 것으로 관찰되었다. 한편 Oil Red O 염색결과 세포내에 lipid droplet이 증가하는 것으로 나타나 지방을 추출하여 처리해본 결과 더욱 뚜렷한 증가를 보였다. 난자의 경우 open culture method를 사용했을 때 수란관 조직 추출액의 독성 효과가 더 크게 나타나 난자의 생존률이 크게 감소하였다.

결 론: 위의 결과로 미루어 소의 수란관 조직 추출액은 생쥐의 난구세포와 난자에 대해 독성 효과를 가지며 이 효과를 나타내는 물질은 수용성 및 지용성을 모두 갖는 것으로 여겨진다.

P-20 The Effects of High Serum Estradiol Concentrations on Implantation and Pregnancy Rates in Fresh in vitro Fertilization-embryo Transfer (IVF-ET) Cycles and Subsequent Frozen-thawed ET Cycles

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Objectives: Several studies have shown significantly lower implantation and pregnancy rates in IVF cycles with high serum estradiol concentrations. The detrimental effects of very high estradiol concentrations on implantation may result from poor embryo quality, lower endometrial receptivity, or a combination of both. But the impact of high estradiol concentrations on the outcome of IVF-ET treatment remains controversial. The purpose of this study was to examine the effects of high serum estradiol concentrations on the day of human chorionic gonadotropin (hCG) administration on implantation and pregnancy rates.

Materials and Methods: A total of 523 women aged <40 years who were undergoing their first IVF cycle were evaluated retrospectively. Serum estradiol concentrations on the day of hCG administration were categorized into three groups: group A < 2,500 pg/ml; group B 2,500~5,000 pg/ml; group C > 5,000 pg/ml. Ovarian stimulation was performed with flare up protocol using gonadotropin releasing hormone agonist

(GnRH-a) and rhFSH (Puregon[®], Organon, Netherland). The outcome of IVF-ET program were analysed using the statistical package for social sciences (SPSS).

Results: In fresh cycles, clinical pregnancy rate per embryo transfer in group C showed decreasing tendency compared to group A and B (17.9 versus 27.1, 28.5% respectively) and implantation rate was 6.1%. However there was no statistical significance. In frozen-thawed embryo transfer cycles, implantation rates in group A, B and C were similar (10.1, 11.7 and 10.1% respectively) and the pregnancy rates were also comparable in all groups.

Conclusions: The implantation rate and pregnancy rate tend to decrease in IVF cycles with high E₂ but in the subsequent frozen-thawed embryo transfer cycle, implantation rate was not impaired. Therefore, our results suggest that the reduced implantation in high E₂ was probably due to adverse endometrial environment.

P-21 Intracellular Free Calcium and Intracellular pH during Compaction of Preimplantation Mouse Embryos *in vitro*

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Introduction: Compaction of 8-cell in the mouse embryo marks the beginning of differentiation in the preimplantation. During the compaction, a role of PKC has been shown to initiate of compaction in the mouse embryo. This study was to examine the influence of PMA, PKC activator, on precompaction of 4-cell embryo and seems to influence the metabolism in this precompaction. In this study it was aimed to measure intracellular calcium and pH in the 4-cell embryos by confocal laser scanning microscope. At precompaction, intracellular calcium and intracellular pH measurement by confocal laser scanning microscope.

Materials and Methods:

- 1) Collection of 4-cell (post hCG 56 hour) mouse embryo on the development and observation after PMA treatment for 2 hour
- 2) Intracellular calcium was determined confocal laser scanning microscope using the calcium sensitive dye fluo 3-AM
- 3) Intracellular pH was determined confocal laser scanning microscope using the pH-sensitive dye SNARF 1-AM
- 4) Chromosomal distributional pattern by Hoechst staining was also examined

Results: 4-cell embryos of mouse was treated with the various concentrations of PMA. After 15 minutes incubation precompaction was induced by 10 and 100 nM. The 10 nM PMA treated group showed significantly higher compaction than that in the control groups. Intracellular calcium transient two times. During the PMA 10 nM treatment for 2 hour, intracellular pH change was observed but it is not known whether the pH change goes up or down. At the precompaction induced by 10 nM PMA treatment, 4 nucleus of 4 blastomere were located in the center of the embryo in which 4 nucleus seem to stick together.