

(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 pre-compaction, 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.

Conclusions: Present study showed that precompaction could be induced by the treatment of 10 nM PMA during 2 hour culture. This precompaction seems to be some relationships with high intracellular calcium concentration with two times of intracellular calcium transients. It also showed that during the precompaction pH changes were observed but it is not confirmed whether it goes up or down.

P-22 생쥐난자의 체외성숙에 미치는 Superoxide Dismutase (SOD)의 영향

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목 적: 체외배양 (*in vitro* culture)시 체내 (*in vivo*) 환경보다 대기에 노출되면서 reactive oxygen species (ROS)가 증가한다. ROS는 산소의 비정상적인 환원에 의해 형성되는데 그 종류에는 superoxide radicals ($O_2^{\cdot -}$), hydroxyl radicals (OH^{\cdot}), hydrogen peroxide (H_2O_2)가 있다. 그러나 생명체에는 superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase/reductase system을 가지고 있어 ROS로부터 스스로 보호한다. 그 중 강한 독성을 가지는 superoxide radicals를 분해하는 SOD가 생쥐난자 체외성숙과 산소 농도에 어떻게 영향을 미치는지 알아보려고 한다.

대상 및 방법: ICR 계통의 3주된 암컷에 5I.U.의 PMSG주사 44시간 후 난자를 얻어 21% O_2 환경과 5% O_2 환경에서 SOD 0, 5, 50, 500, 5,000 unit, CAT 10I.U., hypoxanthine 2 mM를 처리한 후 3시간 후에 핵막붕괴를, 17시간 후에 제1극체 형성여부를 관찰하였다. 또한 21% O_2 환경과 5% O_2 환경에서 SOD 농도별로 3시간, 17시간 배양한 후 confocal laser scanning을 이용하여 난자 내 ROS의 양을 측정하였다.

결 과: SOD를 농도별로 처리한 후 21% O_2 환경에서 50 unit를 처리한 경우 핵막붕괴율이 높았고, 50, 500 unit에서 제1극체 형성율이 유의하게 높게 나타났다 ($p < 0.05$). 5% O_2 환경에서는 유의한 차이를 보이지 않았다. 5,000 unit를 처리한 경우 제1극체 형성율이 모두 유의하게 낮게 나타났다 ($p < 0.005$). SOD와 CAT를 처리한 후 21% O_2 환경에서 실험군 모두 핵막붕괴율이 유의하게 높게 나타났고 ($p < 0.05$), SOD 5, 50 unit 처리군에서 제1극체 형성율이 매우 유의하게 높게 ($p < 0.005$), 500 unit에서도 유의하게 높게 나타났다 ($p < 0.05$). 5% O_2 환경에서 핵막붕괴율이 큰 차이가 없었으나 제1극체 형성은 모두 매우 유의하게 낮게 나타났다 ($p < 0.005$). SOD와 hypoxanthine을 처리한 후 21% O_2 환경에서 실험군 모두 핵막붕괴율이 모두 유의하게 높게 나타났고 ($p < 0.05$), 제1극체 형성율도 모두 유의하게 높게 나타났다 ($p < 0.05$). 5% O_2 환경에서 핵막붕괴율과 제1극체 형성율 모두 유의한 차이가 나타나지 않았다. 21% O_2 환경에서 3시간 배양한 후 ROS를 측정한 결과 SOD 전체 처리군 모두 유의하게 낮게 나타났고 ($p < 0.05$), 17시간 배양 후에도 SOD 전체 처리군 모두 유의하게 낮게 나타났다 ($p < 0.05$). 5% O_2 환경에서 3시간 배양한 후 SOD 5,000 unit를 처리한 경우만 유의하게 낮게 나타났고 ($p < 0.05$), 17시간 배양한 후 5,000 unit를 처리한 경우 유의하게 낮게 ($p > 0.05$), 50, 500 unit를 처리한 경우 매우 유의하게 낮게 나타났다 ($p < 0.005$).

결 론: SOD는 21% O_2 환경에서 ROS를 감소시켜 난자 성숙율을 증가시킨다는 것을 알 수 있었고, 5% O_2 환경에서는 SOD의 영향력이 거의 없었다.