

cultured monolayer of endometrium. Addition of EGF to the growth media modulate the duration of endometrium to reach confluence, but there were no microscopic differences after confluence between endometrium cultured with EGF and endometrium without EGF. However, EGF influence the trophoblast-endometrium interaction in vitro. Within 12 hr of culture with EGF, cytotrophoblast cells were begin to adhere to form the clups of cells. After 28 hr culture, endometrial stromal layer began to detach from the bottom of dishes and After 28 hr culture, endometrial stromal layer began stromal layers were destructed. However, endometrial epithelial layers were intact until 48 hr culture with cytotrophoblast. Three out of 21 samples of stromal layer showed about 50% destruction. Collagenase (0.1%) aaded to the endometrial monolayer destructed both stromal and glandular epithelium within 24 hrs. These in vitro interaction between cytotrophoblasts and endometrium suggests the possibility that EGF is playing role in implantation process and embryo may invade glandular epithelium and stromal layer using different mechanisms.

- 22 -

### **Pinopodes formation in the poor and good responders in human IVF program.**

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Despite progress in human IVF, the majority of IVF attempts remain unsuccessful, most likely on the basis of implantation failure. If we were able to manage the implantation window, we could significantly improve implantation and pregnancy rates in human IVF. The present study was conducted to evaluate the endometrial development in IVF patients related to their plasma level of estradiol(E2) on the day of hCG injection. Patients stimulated by either FSH/hMG/hCG or GnRHa/FSH/hCG were included in this study. Included patients were grouped according to the plasma level of E2 measured on the day of hCG administration as the poor responders(n=6, E2<600 pg/ml) and good responders (n=6, E2≥600 pg/ml). Endometrial biopsy was accomplished two days after oocyte retrieval on the tentative day of embryo transfer in patients who had no embryos available for transfer due to fertilization failure of the all oocytes aspirated. Two-dimensional structure of the endometrium was analyzed by hematoxylin and eosin staining, while three-dimensional structure, pinopodes formation, of the endometrium was assessed by examining through scanning electron microscopy (SEM). Half of the biopsied endometrium was fixed in 10% formalin and processed further for paraffin section. Luteal phase assessment was performed by dating the endometrium according to the standard criteria by Noyes et al. The other half of the biopsied endometrium was rinsed thoroughly with saline and immersed in 2.5% (w/v) glutaraldehyde containing 2% paraformaldehyde solution in PBS. Specimen was fixed in 1% (w/v) osmium tetroxide and dried in a critical-point drier, and then examined by SEM after coating with gold palladium. Mean level of plasma E2 was 395.2 ± 66.3 and 1328.0 ± 215.2 pg/ml in the poor and good responders,

respectively. Greater percentage of simple tubular glands were found in the poor responders. Even if we were able to find consistency in gland and stroma status in the group, it was difficult to judge endometrial development according to dating only.

Less developed pinopodes with long and erect microvilli were found in the poor responders. Results from the present study indicate that endometrial development is delayed in poor responders than the good responders. The most important finding value and dating results later in our study. The lag in endometrial development allows us to predict that the implantation window in poor responders will be delayed than in good responders. It would be worthy to investigate the meaning of this delayed implantation window in poor responders, considering that embryos at the similar stages(2-4 cell)are transferred to patient's uterus in both groups.

대한 정확한 기작은 아직 알려지지 않고 있다. Cortisol은 난포액 내에 다량 존재하며 난포 세포에 수용체가 있는 것으로 보고되고 있다. 이러한 부신 호르몬은 난소의 기능에 직접 영향을 미치는 것으로 알려지고 있으며, 특히 난포 세포의 steroidogenesis에 관여하는 것으로 보고되고 있다. 한편 glucocorticoid는 흉선 세포의 대표적인 apoptogenic agent로써 흉선 세포의 apoptosis에 대한 많은 연구가 있어 왔고 난포 세포에서도 같은 효과 있을 것으로 생각하여 본 실험을 시행하였다.

난자 채취시 얻은 난포 세포를 40% percoll을 처리하여 혈구 세포를 제거한 후 10% FBS를 포함한 Ham's F-10에서 배양하였다. 배양된 난포 세포에 FSH, hCG, dexamethasone(DEX), buserelin등을 각각 또는 함께 처리하여 각 호르몬에 대한 estradiol(E2)와 progesterone(P4)의 농도의 변화를 조사하였다. 그리고 배양 접시 바닥에 붙어 있는 세포의 수를 계산함으로써 배양시간에 따른 생존률을 조사하였다. 배양 마지막 날 배양된 난포 세포를 acridine orange로 염색하고 세포의 핵을 관찰함으로써 apoptosis 진행 정도를 조사하였다.

GnRH-a는 농도 의존적으로 배양중인 난포 세포의 E2와 P4의 생성을 감소 시켰다. 또한 FSH는 GnRH-a의 작용을 억제하였으나 hCG는 영향이 없었다. DEX는 세포의 생존률을 감소시켜 E2와 P4의 생성을 감소시켰으며 FSH에 의해 극복되었다. 이상의 결과에서 GnRH-a는 직접 난포 세포의 steroidogenesis를 억제하지만 세포의 apoptosis와는 직접적인 연관은 없으므로 사료된다. 반면 DEX는 난포 세포의 apoptosis를 유발시켜 E2와 P4 생성을 감소시키는 것으로 사료된다.

- 23 -

## 배양된 배란 전 난포 세포의 apoptosis와 steroidogenesis에 미치는 GnRH-agonist와 cortisol의 영향

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사람의 배란 전 난포 세포에서 GnRH에 대한 수용체의 존재가 확인되면서, GnRH와 그 유도체(GnRH-a)등이 사람의 난포 세포와 황체 세포에 미치는 영향에 대하여 많은 연구가 있어 왔다. GnRH는 일반적으로 난포 세포의 steroidogenesis에 관여하는 것으로 알려지고 있으며, 또한 난포 세포의 apoptosis에 직접 작용을 하는 것으로 보고되고 있다. 그러나 그것에