varying concentrations of hydrosalpinx fluid to evaluate if this fluid affects trophoblast cell proliferation *in vitro*.

Design: 3-day proliferation assay of trophoblast cell in response to hydrosalpingeal fluid from 10 patients undergoing laparoscopy was performed. We also checked the levels of several cytokines (INF-γ, TNF-α, IL-10, IL-6) in this fluid.

Materials and Methods: Hydrosalpinx fluid was aspirated during laparoscopy. All samples were centrifuged at 10,000 RPM for 10 minutes to remove cellular debris and frozen at 20℃ until analysis. Trophoblast cell (Jeg-3 choriocarcinoma cell line; ATTC, Bethesda, MD) proliferation in vitro was determined by a colorimetric immunoassay (Boehringer Mannheim), based on the measurement of BrdU incorporation during DNA synthesis, using a kit. The optical absorbance of the samples was measured in an ELISA reader at 450 nm. Cytokines were assayed by a two step sandwich enzyme immunoassay technique (Biosource, CA; lower limit of sensitivity, 4 pg/mL for IFN-γ, 1 pg/mL for TNF-α, 2 pg/mL for IL-6, 5 pg/mL for IL-10). Data are presented as mean±SEM. Statistical analysis was performed by regression analysis.

Results: Samples from 7 out of 10 patients significantly suppressed trophoblast proliferation in a dose dependent manner ($r=-0.0673$, $p<0.05$). Trophoblast cell proliferation was assayed in the presence of six different concentrations of hydrosalpinx fluid (0%, 25%, 50%, 75%, 90%, 100%). IFN-γ and IL-6 were present in 2 out of 10 HF samples with a mean concentration of 10.45 pg/ml±0.95 pg/ml and 0.814 pg/mL±0.59 pg/mL. IL-10 was present in 4 out of 10 samples with concentration of 2.83 pg/mL±1.27 pg/mL. TNF-α was checked in all samples, with a mean concentation of 13.12 pg/mL±1.10 pg/mL. The mean concentration of TNF-α was greater in the hydrosalpinx fluid from suppressor group than nonsuppressor group (14.74 pg/mL±1.05 pg/mL versus 9.33 pg/mL±0.55 pg/mL, respectively).

Conclusion: In our study, fallopian tube fluid from the majority of women with hydrosalpinx significantly inhibited trophoblast proliferation in vitro model system. Fallopian tube fluid may play a similar role in vivo. Given the possible role of cytokines in the regulation of pregnancy, and maintenance of a proper hormonal milieu, we postulate that high levels of the Th1 cytokine, TNF-α, may represent a potential mechanism for early implantation failure in women with hydrosalpinx.

P-27 Effect of Hydrosalpingeal Fluid on the Implantation in-vitro in a Murine Model

Mi Kyoung Koong[1], Jin Hyun Jun[2], Chun Kyu Lim[2] and Inn Soo Kang[1]

Department Ob/Gyn[1], Laboratory of Reproductive Biology and Infertility[2] Samsung Cheil Hospital, Sungkyunkwan University School of Medicine

Hydrosalpinx affects the clinical outcome of human IVF-ET treatments. However, the mechanism of negative impact on the successful implantation and pregnancy is not fully explained. In our previous study, hydrosalpingeal fluid (HSF) has a mild adverse effect on the early embryonic development of mouse embryos. The aim of this study was to investigate the effect of HSF on

the outgrowth of trophoblasts using mouse embryos. The HSF was collected from eight patients with hydrosalpinx undergoing salpingoneostomy. The fluid was centrifuged and supernatant was stored at -20℃ before use. The mouse blastocysts were obtained from in-vitro cultured 2-cell embryos of superovulated ICR mouse. All blastocysts were treated with 0.5% pronase E to remove zona pellucida, which was for elimination of its affect on implantation process. The five different kinds of culture media were used: Ham's F-10 only (group I), Ham's F-10 media with 0.5% fetal bovine serum (FBS) (group II), 50% HSF with 0.5% FBS-Ham's F-10 (group III), 100% HSF with 0.5% FBS (group IV), and 100% HSF (group V). The blastocysts were randomly allocated to these five media and cultured for 48 hours. The outgrowth of trophoblasts was identified when primary giant trophoblasts were visible around the attachment site following observation under phase-contrast microscope and the surface area of outgrowth was calculated by image analysing system. The outgrowth rates of blastcysts in group I, II, III, IV and V were 0%, 98.9%, 77.5%, 40.4% and 10.0%, respectively. The outgrowth areas of trophoblasts in the media containing HSF (group III, IV, V) were significantly smaller than group II ($p<0.01$). According to the results of this study, the HSF has an inhibitory effect on the outgrowth of trophoblasts. Therefore, we suggest that the HSF may affect the implantation process and the correction of hydrosalpinx before IVF-ET program may be needed to increase the pregnancy rate.

P-28 정자 형성 이상을 보이는 남성 불임 환자에서 정상과 달리 발현되는 새로운 유전자 검색

성균관대학교 의과대학 삼성제일병원 생식생물학 및 불임연구실[1], 비뇨기과[2], 산부인과 불임클리닉[3]

송견지[1] · 박용석[1] · 이형송[1] · 김정욱[1] · 이유식[2] · 서주태[2] · 김종현[2] · 강인수[3]

서 론: 최근 난자내 정자 직접 주입법 (ISCI)와 고환 정자 추출법 (TESE)의 개발 및 이용으로 남성 불임 환자도 아기를 갖을 수 있게 되었으나 아직까지 불임의 원인 규명에 대한 연구는 미비한 실정이다.

정자형성과정은 여러 호르몬의 영향을 받으며 많은 유전자들에 의해 조절된다고 알려져 있다. 그러므로 정자형성에 이상이 있는 환자는 정상과 다른 유전자 발현 양상을 보일 것으로 예상된다. 본 연구는 differental display RT-PCR 방법을 이용하여 비정상적인 정자 형성 과정에서 다르게 발현되는 새로운 유전자들을 검색하고자 한다.

대상 및 방법: Sertoli cell only syndrom (SCO), maturation arrest와 normal spermatogenesis로 진단 받은 무정자증환자군 각 2 명에서 고환 조직을 얻었다. 조직내 total RNA를 추출하여 정량하였다. Diffrenetial display RT-PCR 방법을 이용하여 각 군에서 다르게 발현되는 유전자 band를 분리해 내어 염기서열 분석을 실시하였다.

결 과: 각 환자군에서 다르게 발현되는 유전자 17개를 확인하였으로 이중 8개는 SCO환자군에서만 특이적으로 관찰되는 유전자 (Sco 1 - Sco 8)였으며, 9개는 Normal spermatogenesis군에서만 관찰되는 유전자 (Nor 1 - Nor 9)였다. 17개의 유전자 절편중 현재까지 7개의 유전자 절편이 성공적으로 cloning되어 염기서열 분석을 실시하였다. Sco 1, Sco 2 그리고 Nor 8 유전자 조각은 지금까지 보고된 바 없는 새로운 염기서열임을 알수 있었다. Nor 1은