expression in mouse ovarian tissue.

Methods: Cryopreservation of mouse ovarian tissue was used by slow freezing method. The mRNA level of HSP90 expression in both fresh and cryopreserved mouse ovarian tissue was analysed by semi-quantitative RT-PCR. The protein expression of HSP90 was evaluated by Western blot analysis and immunohistochemistry.

Results: The two subunits of HSP90 (hsp90α and hsp90β) mRNA were expressed in both fresh and cryopreserved mouse ovarian tissue. The amount of hsp90α and hsp90β mRNA was increased in cryopreserved ovarian tissue after 30 minutes and 1 hour of thawing and incubation in vitro. The amount of HSP90 protein was increased in the cryopreserved ovarian tissue after 6 hours of the incubation in Western blot analysis. In immunohistochemical study, HSP90 was localized in cytoplasm of oocyte and granulosa cell from primary follicles and preantral follicles. Significant level of immunoreactive HSP90 was detected in theca cells while only traceable amount was found in ovarian epithelial cells.

Conclusions: The present study demonstrates the increase of HSP90 expression in the cryopreserved mouse ovarian tissue. It is suggested that HSP90 may play a role in the protective and/or recovery mechanism from the damage by cryopreservaiton.

B-3 The mRNA Expression Patterns of LH Receptor in Human Leutenized Granulosa Cells

Lee JB[1,2], Kim ES[1], Kim MH[1], Lee KH[1], Roh SI[1], Kim MK[2], Yoon YD[2], Do BR[1], Yoon HS[1,2]

Infertility Research Center, MizMedi Hospital[1]

Department of Life Science, College of Natural Sciences, Hanyang University[2]

Objectives: We investigated the expression patterns of LH receptor (LH-R) in human leutenized granulosa cell (LGC)s and analyzed the relationship between LH-R mRNA expression and pregnancy rates, number of retrieved oocytes and oocyte quality, retrospectively.

Materials and Methods: LGCs were prepared at the time of oocyte retrieval from the patients undergoing IVF-ET program. The patients were divided into two groups: Group I (n=8) is poor responder (oocytes≦4ea), Group II (n=40) is normal responder (oocytes > 4ea). After the extraction of total RNA, semiquantitative RT-PCR of LH-R mRNA was performed with same amount of RNA (30 μg/ml) and cDNA in these two groups and LH-R expression was quantified individually. The relative values of LH-R mRNA expression is represented as the results of LH-R/β-actin. Statistical analysis was performed using χ^2 test, student's t test and Pearson correlation.

Results: In Group II, LH-R expression was slightly stronger than in group I (0.516 vs 0.713), and also pregnancy rate was higher than group I (12.5% vs 48.7%, p=0.059), but there was no significant difference. LH-R expression was gradually increased when the number of retrieved oocytes was increased (p=0.014), but it was not clear that the relationship between LH-R and number of growing follicles. Also oocyte

quality was not related with LH-R. When LH-R expression was compared with pure FSH only group and FSH combined with hMG group in the ovarian stimulation protocol, LH-R expression was significantly higher in hMG combined group than FSH only group (p=0.028).

Conclusions: This data suggest that the expression of LH-R mRNA is important to the ovarian function related with responsibility of gonadotrophinin human folliculogenesis. The confirmation of the expression patterns of gonadotrophin receptor mRNA in LGCs will be required to investigate the relationship between LH-R and FSH-R in human folliculogenesis.

B-4 Cumulus Free 생쥐 성숙난의 Vitrification 후 Simple Media에서의 수정 및 배 발달

고려대학교병원 산부인과학교실

오지현 · 이정재 · 김 탁 · 김선행

목 적: 본 실험은 vitrification 과정을 거친 cumulus free 생쥐 성숙난을 simple media에 배양하였을 때 수정과 배 발달, 나아가 핵수가 어떻게 나타나는지 살펴보고 그 효율성을 가늠해 보고자 실시하였다.

대상 및 방법: 실험에 사용된 성숙난의 회수는 5~6주령된 ICR 암컷 생쥐에 PMSG와 hCG를 48시간 간격으로 5 IU씩 주사하고, hCG 주사 후 14~15시간째 oocytes-cumulus-complex를 난관 팽대부로 부터 채취하여 얻었으며, cumulus 제거를 위해 0.1% hyaluronidase 용액을 이용하였다. 회수된 성숙난은 대조군과 실험군으로 나누어, 실험군은 1.5 M EG과 5.5 M EG+1 M Sucrose 용액에서 각각 2.5분과 20초간 노출시킨 후 EM grid로 옮겨 바로 LN_2에 침지하는 방법으로 vitrification을 실시하였으며 5~7일간 냉동 보관하였다. 동결 난자의 융해는 1 M, 0.5 M, 0.25 M, 0.125 M Sucrose 용액에 차례로 2.5분씩 처리하는 방법을 사용하였고 1시간 정도 전 배양시켜 실험에 공시하였다. 두 군의 IVF를 위한 정자는 12주령 이상 된 ICR 수컷 생쥐의 정소상체 미부로 부터 회수하였고 수정능력 획득을 위해 1.5~2시간의 전 배양을 실시하였다. 수정 및 배 발달은 T6 배양액에 0.4% BSA를 첨가하여 사용하였다.

결 과: 수정율에 있어 대조군은 198개중 184개가 수정되어 92.9%를 나타내었으며, 실험군은 먼저 147개 중 113개가 융해 후 생존하여 76.9%의 생존율을 나타내었고 이중 90개가 수정되어 79.6%의 수정율을 나타냈다. 이는 대조군과 비교할 때 매우 높은 유의차을 보여준다 ($p<0.005$). 배 발달율에 있어서 대조군은 2C, 4C, 8C 및 Morula가 각각 92.4, 85.9, 82.6 및 82.6%였고 실험군은 91.1, 84.4, 78.9 및 76.7%로 두 군 사이에 유의차는 없었으나 배반포율에 있어서는 대조군이 76.1% 실험군이 63.3%로 유의차가 인정되었다 ($p<0.05$). 핵수에 있어서도 대조군이 63.5개, 실험군이 58.9개로 대조군에 비해 실험군이 유의하게 적었다 ($p<0.05$).

결 론: 본 실험의 결과로 볼 때 수정율, 배반포율 및 핵수에서 두 군 사이에 유의차는 인정되었으나 simple media를 이용한 vitrification은 생쥐 성숙난의 동결 방법으로서 매우 효과적임을 알 수 있었다. 나아가 공시 난자를 cumulus enclosed 성숙난을 이용하거나 동결과정에서 야기되는 투명대 경화 현상 및 세포질 내 기관의 불안정성 등을 방지할 수 있는 물질의 첨가 등이 이루어진다면 더욱 향상된 결과를 얻을 수 있을 것으로 사료된다.