10 *In Vitro/In Vivo* Development of Mouse Oocytes Vitrified by EFS

Myo Kyung Kim, Eun Young Kim, Bong Kyung Yi, Hyeon Sook Lee, San Hyun Yoon*, Sepill Park, Kil Saeng Chung and Jin Ho Lim***

*Maria Infertility Medical Institute (마리아기초의학연구소), *Maria OB/GYN, Seoul*
***College of Animal Husbandry, Kon-Kuk University*

Mouse oocytes were cryopreserved by the vitrification method using EFS30, 35, 40 (30, 35, 40% ethylene glycol, 0.5M sucrose and 18% ficoll in M2) and were examined the effects of dilution methods on the rate of *in vitro* development. After thawing and dilution of the cryoprotectant, oocytes of normal morphology were inseminated, the rates of fertilization, development *in vitro* and cell number of blastocysts were examined. For vitrification and toxicity test, oocytes were exposed to M2 solution containing 10% ethylene glycol for 10 min. and transferred to EFS for 30 sec, they were then cooled rapidly in liquid nitrogen. The highest cleavage rates were obtained in EFS35 (40.5%) and 2-step dilution method (50.0%) when oocytes were exposed to M2 containing 0.5 M sucrose and then fresh M2 for 5 min., respectively. The development rate of vitrified-thawed oocytes to the blastocysts stage after *in vitro* fertilization (92.9%) was not significantly different compared to that of control (80.0%). Also, the mean number of cells per blastocysts (85.6 ± 15.3) was similar to that of the control (101 ± 18.6). Development *in vivo* was assessed by transferring blastocysts derived from vitrfied-thawed oocytes into the uterine horns of day 3 pseudogregnant female recipients. Transfer of the blastocysts resulted in fetal development (44.9%) and implantation rates (81.2%) similar to those of the control (50.0, 75.0%). These results suggested that mouse oocytes could be vitrified using cryopreservation solution (EFS35) based on ethlyene glycol.

11 A Study of HSP70A and HSP70B Gene Expression in Testis Tissue of Azoospermia Men Without DAZ Deletion; HSP70B as a maturation arrest factor in spermatogenesis

Ho-Joon Lee[1], Gyun Jee Song[1], Hyoung-Song Lee[1], Young-Seog Park[1], Hye-Kyung Byun[1], Jong Hyun Kim[2], You Sik Lee[2], Keun Jae Ryu[3], Ji Hong Song[3] and Ju Tae Seo[2]

Infertility Research Laboratory[1], Department of Urology[2], Department of Obstetrics and Gynecology[3], Samsung Cheil Hospital and Women's Healthcare Center, College of Medicine[3], Sung Kyun Kwan University

Spermatogenesis is known to be regulated by a number of genes and several factors such as hormones, growth factors, cytokines and others. This study was done to evaluate the relationship between HSPs and DAZ genes in human spermatogenesis; we observed the expression pattern of HSP gene in azoospermia men with DAZ gene that regulated the gene expression related with

human spermatogenesis. RT-PCR method was used to detect DAZ, HSP70A, and HSP70B transcripts in all RNA samples. Total RNA was extracted from 22 testis tissues using TRIZOL reagent. cDNAs were synthesized with reverse transcriptase, AMV. All PCR reaction were performed on a PCR themocycler with DAZ, HSP70A, and HSP70B-specific primers. PCR products were subjected to electrophoresis on a 2% agarose gel, stained with ethidium bromide, and photographed. Semen analysis, karyotyping and testis histology were performed. DAZ gene, known as a candidate gene of azoospermia factor (AZF), was deleted in 3 of 21 patients (16%). To evaluate the only effects of HSPs in this patients, 3 DAZ deleted cases were removed. HSP70A gene was detected in all patients tested. But HSP70B gene was not detected in 11/18 (61%) without DAZ deletion. In 13 non-obstructive azoospermia patients, 4 of 7 Sertoli cell only, 3 of 5 hypospermatogenesis, 1 of 1 maturation arrest patients have no HSP70B transcript. Of those patients, 8 patient were identified various types of sperm such as immature sperm and mature sperm. Whereas HSP70B gene didn't express in 4 of 5 obstructive azoospermia, who have mature spermatozoa in testicular biopsies. This results may be indicated that expression of HSP70B is related with spermatogenesis, especially, and sperm maturation process. In conclusion, HSP70B as well as DAZ gene seem to be involved causing spermatogenic failure. We suggest that HSP70B palys an important role in spermatogenesis and one of factors induced sperm maturation in human. Therefore, we think that HSP70B may be a maturation arrest factor in human spermatogenesis.

12 무정자증 환자에서 YRRM 과 Y-specific STS (Sequence-tagged sites)의 유전자 분석

포천중문의대 차병원 여성의학연구소 비뇨기과, 산부인과, 유전학 연구실

이숙환 · 김현주 · 한정희 · 조성원 · 엄기붕 · 고정재
곽인평 · 한세열 · 윤태기 · 차광열

원인 불명의 Azoospermia로 인한 남성불임의 빈도는 10% 정도로 결코 낮지 않은 비율을 차지하고 있다. 1976년 Azoospermia 환자에서 Y-염색체 submicroscopic deletion 이 밝혀진 이래 여러 연구에 의해 Y-염색체내에서 spermatogenesis를 control하는 하나 또는 그 이상의 유전자 (AZF, Azoospermia factor)는 interval 6에 해당된다는 것이 알려졌다. 1993년 Ma 등은 Y 염색체 interval 6 내에는 Y-chromosome RNA recognition motif (YRRM)라는 일종의 gene family를 identify 해냈고 infertile men에서 이 YRRM sequence내에 deletion이 있음을 밝혀냈다.

이 gene family가 AZF의 candidate gene일 수 있다는 설은 아직 확인이 되지 않고 있다.

본 연구에서는 Azoospermia 환자에서 Y 염색체의 microdeletion여부를 보기 위해 YRRM sequence 및 interval 6 에 해당되는 13개의 Y-specific STS 부위에서 PCR을 이용했다.

61명의 Azoospermia 환자에서 FSH, LH 등의 hormone analysis 및 testicular biopsy 을 시행하였다. 모두 61명의 Azoospermia 환자 중 PCR 결과 18.03% 에 해당하는 11명에서 deletion을 보였으며 이 중에는 multiple deletion을 나타낸 경우도 있었다.

이와 같이 Azoospermia 환자에서 Y-specific region의 PCR amplification은 infertile men의