

the quality of spermatozoa after cryopreservation either by vitrification with a very fast cooling rate or slow freezing with a programmable freezer.

**Method:** Ejaculates were obtained from 13 male partners during routine infertility investigation. Completely liquefied ejaculates were analyzed according to the WHO criteria by computer-assisted semen analyzer (CASA). Each ejaculate was mixed with TYB-buffered freezing medium and then, divided into two parts; one for vitrification by a direct plunging into liquid nitrogen and the other for slow freezing. Thawing was achieved by plunging of the vials containing frozen sperms into 37°C water. They were analyzed by CASA. In addition, DNA integrity of spermatozoa was examined by TUNEL assay.

**Results:** No significant difference was found in recovery rate of motile fraction between the two methods of cryopreservation (43.7% vs 45.2%, respectively). And also, DNA integrity of spermatozoa has no difference (35.4% vs 31.7%).

**Conclusions:** Sperm vitrification method can be an alternative for the cryopreservation of ejaculated human spermatozoa, which can avoid the use of the freezing equipment. The sperm qualities were similar after vitrification vs slow freezing methods. However, considering convenience of the procedure, vitrification is more favored for cryopreservation of spermatozoa.

## P-18 Relationship between Genetic Polymorphisms of FSH Receptor and Outcome of Controlled Ovarian Stimulation

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**Background & Objectives:** Controlled ovarian hyperstimulation (COH) using exogenous FSH is a widely used treatment in most assisted reproduction techniques. FSH triggers the maturation of follicles and plays a pivotal role in the recruitment of the dominant follicle. These important actions of FSH are mediated by the FSH receptor. In this study, we evaluated the association of FSH receptor gene polymorphisms with outcome of COH in patients undergoing IVF.

**Method:** Genomic DNA was extracted from peripheral blood of 1020 women, including 551 women were undergoing COH. We investigated the frequency of FSH receptor variants, Thr307Ala (T/A) and Asn680Ser (N/S) polymorphisms by using polymerase chain reactions and restriction fragment length polymorphism analysis. Their clinical outcome such as basal FSH level, estradiol level at administration of hCG, dosage of FSH treated, number of retrieved oocytes and pregnancy rates were compared related to their genotypes.

**Results:** In a population of 551 Korean women, the frequency of TT/NN, TA/NS and AA/SS for the variant Thr307Ala and Asn680Ser was 44.80%, 41.96% and 10.49%, respectively. The frequency of the unlinked genotypes, TA/NN, TT/NS, AA/NN and AA/NS were 1.86%, 0.29%, 0.20% and 0.29%, respec-

tively. There was no significant difference in the basal FSH level, dosage of FSH treated and pregnancy rate among the COH-IVF patients with different genotypes. However, the proportion of good responder, which is higher estradiol level (> 2,000 pmol/L) at administraton of hCG and number of retrieved oocytes (> 10 oocytes), in AA/SS genotype (65.9% and 68.2%) was higher than those of TT/NN (51.1% and 57.3%) and TA/NS (50.2% and 59.2%).

**Conclusions:** The FSH receptor gene polymorphism (Thr307Ala and Asn680Ser) may be associated with outcome of controlled ovarian stimulation in patients undergoing ART program. Our finding is contrast to the reports with other ethnic populations that AA/SS genotypes highly responded to FSH treatment. Our data should be substantiated by the further studies of FSH receptor genotyping in the Korean population.

## P-19 IGFs가 생쥐 수정란에서 발현되는 Apoptosis에 미치는 영향

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**Background & Objectives:** 체외에서 수정된 수정란은 체내에서 수정된 수정란에 비하여 apoptosis가 높게 나타나고, 발생하는 속도가 느리다. 이러한 양상은 수정란에 의해 생산되는 여러가지 성장인자들이 잘 발현되지 않는다는 것을 시사한다. 수정환경의 차이, 즉 체외와 체내에서의 수정은 포배기까지 발생하는 동안에 IGF-I과 -II 발현에서 apoptosis의 차이를 나타냈다. 이러한 결과는 내재적 성장인자의 높은 발현으로 인하여 autocrine pathway에 의하여 apoptosis의 억제를 유발한 것으로 사려되었다. 따라서 본 연구는, 체외수정과 배양시 첨가된 IGF-I과 -II가 수정란의 발생과 apoptosis의 억제에 어떠한 영향을 미치는 지를 체내수정과 비교하였다.

**Method:** 5주령의 BD F1 생쥐에게 7.5 IU의 PMSG를 주사하고, 48시간 후에 7.5 IU의 HCG를 주사하여 다배란을 유도하였다. 체내수정은 동종의 웅성생쥐와 합사하여 유도하였으며, 0.4% (w/v) BSA를 함유한 MTF배양액으로 포배기까지 배양하였다. 체외수정은 HCG를 주사한 14시간 후에 난자를 회수한 후, IGFs 비첨가군과 첨가군 (10 ng/ml)으로 나누어 각각의 그룹에 정자를 첨가함으로써 유도하였다. 수정확인은 2-세포기 배아로서 판정하였으며, 각 실험군은 0.4% (w/v) BSA를 함유한 MTF배양액에 수정시와 동일한 농도의 IGFs를 첨가하여 포배기까지 배양하였다. 두 군간에서 세포수의 차이는 포배기배아를 Hoechst로 염색한 후 관찰하였으며, apoptosis의 빈도는 TUNEL방법을 사용하여 형광현미경하에서 확인하였다.

**Results:** 수정율을 비교한 결과, IGFs 비첨가군 (64.0%)이 IGFs 첨가군에 비하여 낮게 나타났으나 ( $p < 0.05$ ), IGFs 첨가군간 (IGF-I: 74.2; IGF-II: 71.2; IGF-I & II: 73.0)의 차이점은 없었다. 포배기배아의 발생율을 비교한 결과, 체내에서 수정한 군 (97.7%)에 비하여 체외에서 수정한 군이 낮게 나타났으며, 비첨가군 (78.2%)에 비하여 IGFs 첨가군 (IGF-I: 87.0; IGF-II: 88.2; IGF-I & II: 84.9)이 배아의 발생율이 높았으나, IGF-I 또는 -II를 각각 첨가하였을 때나 함께 첨가했을 때의 영향은 없는 것으로 나타났다. 포배기배아에서 총 세포수와 apoptosis의 비율을 비교한 결과, 체외수정군 중 IGFs를 첨가한 군 (IGF-I: