

polymorphism and PCOS is under investigation. Currently, studies of Pro12Ala variant in exon2 and C/T substitution in exon6 in PCOS patients showed that the levels of BMI and leptin were correlated with polymorphisms of exon6. In this study, we investigated the polymorphism of these three genes to determine whether they are associated with PCOS in Korean women of reproductive age.

**Method:** Using restriction fragment length polymorphism (RFLP), the polymorphisms were analyzed in 71 Korean PCOS patients and in 26 control patients.

**Results:** MM type of TNFR2 exon6 (69%) and CT type of INSR (48%) were predominant than other types (TNFR2; MR, RR, INSR; CC, TT). In PPAR- $\gamma$  studies, the C/G polymorphism of exon2 was similar to previous reports, however, that of exon6 showed that the C/T phenotype was predominant than C/C type, which is different from Italians.

**Conclusions:** Interestingly, the predominance for CT type of INSR was not shown with a Korean population, different from the previous report done by other research groups in the United States. We also observed the different predominance of polymorphisms in exon6 against previous report.

## P-60      A Simple, Easy and Efficient Vitrification Method for Cryopreservation of In Vitro Produced Human Embryos

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**Background & Objectives:** This study was to examine the usability of a new vitrification method for cryopreservation of in vitro produced human embryos from IVF-ET program.

**Method:** Human multi-pronuclear (>3PN) embryos were co-cultured with cumulus cells in modified CR1aa medium for 5 to 6 days. In vitro developed blastocysts were collected and exposed in vitrification solution with 2-steps; i) 10% ethylene glycol (EG) and 10% FBS in D-PBS for 5 min. ii) 30% EG, 0.5 M sucrose (S) and 10% FBS in D-PBS for 30 sec. And then embryos were loaded onto our designed minimum volume cooling (MVC) straw and plunged directly into LN<sub>2</sub>. For thawing, MVC straw in LN<sub>2</sub> was quickly transferred into 0.5 MS (and 10% FBS in D-PBS). After recovery, embryos were again transferred into 0.25 MS, 0.125 MS and then finally into 10% FBS in D-PBS for 1 min per each. All treated embryos were transferred onto cumulus cell drop in 10% FBS added CR1aa. 16 hrs after thawing, embryo survival was determined and stained with hoechst 33342 or Live and Dead reagent to check the viability.

**Results:** By simple and easy vitrification and thawing method (freezing for 6min.; thawing for 5 min.), survival of human embryos was indicated high percentage (79.2%, n=24). Also, when optically survived embryos were determined by Live and Dead staining (live=green color, dead= red color), almost of them were confirmed viable.

**Conclusions:** Therefore, using this introduced vitrification method, human embryos can be cryopreserved with simple and efficient.