P-27 Semiquantitative Analysis of Common Deletion in Mitochondrial DNA of Single Human Oocytes

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Background & Objectives: Most cells in the body contain between 100 and 10,000 copies of mitochondrial DNA (mtDNA). This mtDNA plays an essential role in the life cycle via the control of energy production by oxidative phosphorylation. The asymmetric nature of inheritance is exclusively through the female oocyte. In a mature human oocyte, much higher copy numbers (2×100,000) of mtDNA is present to prepare for the energy demands of embryogenesis. There are measurable levels of the so-called "common deletion", which removes 4,977 bp between the ATPase8 and ND5 genes in some oocytes. Not all common deletion becomes pathogenic, but some studies have reported the frequency of deleted mtDNA in oocytes, embryos and have demonstrated that the shift in the mutation decreases with oocyte and embryo development. The purpose of this study was to determine the semi-quantitation of common deletion in oocyte developmental potency.

Method: All procedures were reviewed and approved by Pochon CHA University's Institutional Review Board. Only those oocytes with no evidence of fertilization were used in these experiments. Sixty-seven patients from the CHA IVF-ET program donated a total eight-five unfertilized metaphase II (MII) oocytes derived from mature (Group 1) and immature (Group 2) stage. After removing zona pellucida of oocyte with acidified Tyrode's solution, a single oocyte was placed in 5 μl of lysis buffer to release DNA. Two rounds of PCR preceded by aliquots of two 2.5 μl were performed for common deletion using primers MT-1 (8239-8263) and MT-3 (13551-13575) followed by primers MQ-1 (8421-8441) and MQ-3 (13500-13520). For the purpose of semiquantitation, other site within the oocyte mitochondrial genome, 16s rRNA was also amplified to control the DNA in each sample using primers mt16s-F (2515-2537) and mt16s-R (2984-3003) followed by primres mt16s-1F (2642-2662) and mt16s-1R (2830-2850). A 10 μl aliquot of the PCR product for common deletion and 15 μl aliquot of 16s rRNA were run on a 2% agarose gel, stained with ethidium bromide and photographed. The relative abundance of the PCR products was determined by the Imaging Densitometry and results were expressed as a ratio of band intensity of common deletion divided by 16s rRNA.

Results: A total of 85 unfertilized oocytes from 67 patients were analyzed, in which 42 (49.4%) showed deletion. At developmental stage, the ratio of common deletion divided by 16s rRNA was 24.1553 in Group 2 stage oocytes and 12.5837 in Group 1 stage oocytes. Data were compared with the use of χ_2 and Anova tests. Statistical significance was defined as $p<0.05$.

Conclusions: Although the sample size was too small to conclude definite effects of common deletion, there seemed to be a trend toward decreased amount of common deletion in mature oocytes than in immature oocytes. According to this semi-quantitative analysis, we can hypothesize that the amount of

mtDNA deletion may have effect on the oocyte maturity with subsequent fertilization. This finding is supported by reports of Brenner et al., Keefe et al. who described: the potential role of mtDNA together with the effect of altered oxidative phosphorylation and cellular antioxidant systems on the cytoskeleton, fertilization and subsequent embryo development.

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P-28 Relationship between CYP17, CYP11α, INSR, TNFR and PCOS in a Korean Population

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Background & Objectives: Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age. However, only a few genes have presented the relationship with PCOS. Since genes encoding enzymes in the testosterone synthesis and in the cholesterol side-chain cleavage are implicated in PCOS, we have analyzed the polymorphisms in the promoter of CYP17 gene for the frequency of T to C substitution and in the promoter of CYP11α gene for the (tttta)n repeat to determine whether they are associated with PCOS in Korean women of reproductive age. In addition, we analyzed the polymorphisms of INSR and TNFR-2 to investigate the insulin resistance in hyperandrogenism of PCOS.

Method: Using restriction fragment length polymorphism (RFLP) and microsatellite polymorphism by variable number tandem repeat (VNTR), the polymorphisms were analyzed in 71 Korean PCOS women patients and in 26 control patients.

Results: The allele frequency of the genotype A2A2 for CYP17 was 4 times higher than the one in Greek population with PCOS (48% vs. 8%). In addition, the genotype analysis of PCOS patients for the CYP11α (tttta)n repeat polymorphism revealed 35% 216+ and 65% 216- genotypes, respectively. This is similar to the study performed with British and Greek population. MM type of TNFR2 exon6 (69%) and CT type of INSR (48%) were predominant than other types (TNFR2; MN, NN, INSR; CC, TT).

Conclusions: The difference of the allele frequencies between Korean and other populations for CYP17 and CYP11α suggests that the role of polymorphism may be due to various ethnical backgrounds in PCOS patients. Interestingly, the predominance for CT type of INSR was not shown with a Korean population, different from the previous report done by other research group in the United States.