

## **P-558 Association of folate-pathway gene polymorphisms with pregnancy outcome in recipient women undergoing in vitro fertilization with donor eggs**

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**Introduction:** Folate metabolism disorders have been related with poor fertility outcome, most frequent condition is hyperhomocysteinemia and altered DNA methylation patterns due to genetics variants and depleted dietary intake of folate and B12 related vitamins. Periconceptional folic acid and vitamin B12 supplementation has been largely extended during last decade and we have implemented in recipients undergoing our IVF oocyte donation programs. Genetic condition of recipients has lost interest because of their small contribution in embryo inheritance. However the association of single nucleotide polymorphisms (SNPs) in the one carbon methyl group pathway and in vitro fertilization (IVF) outcome is still unclear. Our aim is to analyze distribution and association of functional SNPs of enzymes related to methyl group metabolism in recipients undergoing IVF treatment in our oocyte donation program with fertile tested donors. For this purpose we decided to study distribution of polymorphic markers of key regulating enzyme of Folate and Homocysteine metabolism MTHFR677 (rs1801133), MTHFR1298 (rs1801131), MTR (rs12749581), CBS (rs5742905) and TCN2776 (rs1801198). Estrogenic metabolism of recipient was assessed for of two relevant SNPs ESR15P (rs2234693) and ESR13P (rs9340799).

**Material and Methods:** A total of 42 recipients without child undergoing IVF treatment in our oocyte donation program were included with average age of 37.8 ( 6.5 years) they were women with at least one previous attempt with own oocyte in IVF treatment. Donors were mothers with children that had provided at least two ongoing pregnancies in no more than 3 embryo transfer in different recipients. Genomic DNA extraction was carried out from buccal swab (Qiagen Mini KIT). Genotyping of SNPs was developed by PCR multiplex amplification and minisequencing (SNaPshot™ ABIPRISM 3130). Hardy-Weinberg equilibrium was assessed for markers in all groups. Chi-squared test was performed for compare differences between recipients and general population frequencies and also between pregnant and non pregnant recipients condition. Logistic regression models were calculated for odds ratio (OR), 95% confidence interval (CI), and corresponding p values performed for assess clinical pregnancy outcome and genotype distribution.

**Results:** Ongoing pregnancy rate were of 52.4%. Distribution of all genotypes were in HW equilibrium except for markers MTHFR C677T (rs1801133) ( $p = 0.049$ ) in non pregnant woman. Higher frequencies of mutant alleles were found in recipients respect general population in markers MTHFR677 (rs1801133) [ $X^2 = 7,639$ ;  $p = 0,0219$ ], MTR (rs12749581) [ $X^2 = 7,639$ ;  $p = 0,0219$ ]. However all mutant alleles of the other markers were overrepresented in recipients without statistical significant results. When comparing clinical pregnancy outcome between recipients, significant differences in genotype frequencies were also found in the MTHFR A1298C (rs1801131) [ $X^2 = 6,615$ ;  $p = 0,0366$ ] in this case mutant allele was present in higher frequency in pregnant recipients. The results show that the recipient group is highly selective confirmed by deviation of the genotypes frequencies for the folate gene polymorphisms studied. Supposedly, due to all women were treated with folate and Vit B12 supplementation, genetic factor should acquired less relevance. In fact 2 pregnant recipients presented 3 mutations for the C677T and A1298C MTHFR polymorphisms (CTCC). This genotype only normally found in fetal samples, are very rare in general populations (0.002). Strong association of the A1298C MTHFR mutated allele (OR = 3.72  $p = 0.0077$  (1.27-10.86) 95% CI) with the pregnancy outcome could be related with the low incidence of T allele in the MTHFR C677T SNP considered as functionally more relevant on enzymatic activity.

**Conclusions:** Folate gene variants present a high incidence in infertile women and could be involved in IVF outcome, although further analysis with higher population need to be completed to extract clinical conclusions. Supported by Grants SAF2008-03314 and PTQ 09-01-00496.

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