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Title:

Influence of 5-HTTLPR variants in the SLC6A4 gene over pregnancy outcomes among recipients of donated eggs.

Abstract Text:**Introduction**

Serotonin is an essential monoamine that plays a role in uterine blood flow, endometrial decidualization and embryo implantation. Serotonergic activity is regulated by serotonin transporter gene (5-HTT) that is occasionally expressed in migratory cells inside endometrial tissue during early pregnancy decidua. A serotonergic local network has been proposed to be an important key player acting locally in female genital tracts and being even more interesting target of study since it has been reported that 5-HT transporter gene (SLC6A4) is expressed in mammalian oocytes and embryos. A specific alleles at an insertion/deletion promoter polymorphism (5-HTT gene-linked polymorphic region, HTTLPR) regulates the transcription of SCLA6A4 gene and activity of 5-HTT in different human cells, the long allele (L) being responsible for increased transcriptional efficiency and higher rate of 5-HT uptake by the 5-HTT. SLC6A4 gene variation suppose an interesting target marker for assess the effect of this serotonergic activity on in vitro fertilization (IVF) treatments outcome. Recipients undergoing egg donation IVF treatment have been selected to study the influence of this polymorphism in treatment success rates and related pregnancy outcome.

Material and Methods

A total of 183 recipients undergoing first IVF treatment at Instituto de Fertilidad Clinica Rincon oocyte donation program with good quality embryo transfer were included. Standardized protocols were applied in donors for ovarian stimulation as well as in recipients for the endometrial preparation. Recipients with uterine and endometrial defects were discarded as well as those women with a recurrent miscarriage failure history. Blood swap were collected and genomic DNA extraction was performed (Qiagen Mini-Kit). Genotyping of SLC6A4 was carried out through PCR amplification achieved in a 2720 ThermalCycler® (Applied Biosystems). Separation of PCR products and subsequent size fragment analysis (ABIPRISM 3130) was performed. Hardy-Weinberg equilibrium was assessed for each group. Fisher exact test was performed to compare genotype distribution and logistic regression models were calculated for odd ratio (OR), 95% confidence interval (CI) for asses possible genotype association with pregnancy

outcome, including chemical pregnancy loss, early pregnancy loss and clinical spontaneous abortion.

Results:

Distribution of genotypes frequencies were in Hardy-Weinberg equilibrium except for non pregnant women. Recessive model for L allele was performed and Fisher exact test showed different genotype distribution [$p=0.00698$] between chemical pregnancy loss group and clinical pregnant recipients (0.79 vs 0.26). Logistic regression model showed a strong association [OR=12.89 $p=0.0007$ (2.47-67.23) 95%] between chemical pregnancy loss and LL genotype. Although recipients of early spontaneous miscarriage group showed higher frequencies of LL genotype too, there were no statistical differences compared to clinical ongoing pregnancy recipients.

Conclusions:

A strong association between long homozygous genotype and the occurrence of early pregnancy loss has been observed. Higher transcriptional profile of L/L SLC6A4 gene variant could influence the regulation of serotonin uptake during decidualization, when the serotonin receptor is occasionally transcribed at low levels. It could affect serotonin extracellular regulation and could be related to impaired events that affects to the normal endometrial development. New findings are necessary to assess functional and clinical impact of serotonergic local network in reproductive outcome. Supported by Grants SAF2008-03314 and PTQ 09-01-00496