The p53 codon 72 single nucleotide polymorphism lacks a significant effect on implantation rate in fresh in vitro fertilization cycles: an analysis of 1,056 patients

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Objective: To determine whether the p53 codon 72 single nucleotide polymorphism, a change of the amino acid arginine (Arg) to proline (Pro) resulting from a single nucleotide mutation of guanine (G) to cytosine (C), has a clinically significant effect on implantation rate in fresh IVF cycles.
Design: Prospective cohort analysis.
Setting: University-affiliated private IVF center.
Patient(s): One thousand fifty-six female patients undergoing fresh nondonor IVF cycles.
Main Outcome Measure(s): Embryo implantation rate.
Result(s): Of the 1,056 patients (2,600 total embryos transferred) undergoing their first IVF cycle, 289 had no implantation events and attempted a second cycle. Of the 289 patients in their second cycle, 72 had no implantation events and attempted a third cycle. The p53 codon 72 single nucleotide polymorphism frequencies in the first cycle (homozygous major allele Arg/Arg [G_G] = 45%, heterozygous allele Arg/Pro [G_C] = 44%, and homozygous minor allele Pro/Pro [C_C] = 11%) did not differ significantly across subsequent IVF cycles. There was no statistically significant difference in embryo implantation rate with respect to the single nucleotide polymorphism.
Conclusion(s): The p53 codon 72 single nucleotide polymorphism lacks a clinically significant effect on embryo implantation rate in patients undergoing fresh nondonor IVF cycles. (Fertil Steril® 2009;92:1290–6. ©2009 by American Society for Reproductive Medicine.)

Key Words: p53, SNP, implantation rate, IVF outcomes, infertility, pregnancy

The number of women undergoing IVF for infertility is increasing, but the parameters that determine whether a given IVF cycle results in a viable pregnancy still largely are undetermined (1). An IVF cycle can fail for multiple reasons based on the quality of the embryo and receptivity of the endometrium. Complicating matters further, the maternal-fetal interface formation may depend on specific combinations of maternal and embryonic parameters.

The relatively recent increase in human genomic knowledge and DNA technology efficiency opened new opportunities for exploring genetic factors leading to implantation failure, such as the effect of common variations of genes in the population resulting from a mutation in a single DNA base called single nucleotide polymorphisms (SNPs). Well more than 3 million SNPs were identified and sequenced in the human population by the HapMap Project (2), but how they relate to different diseases and how they interact with each other is still largely unknown. Single nucleotide polymorphisms that have been studied for human reproduction include methylenetetrahydrofolate reductase (3, 4), plasminogen-activator inhibitor-1 (5), P receptor (6), FSH receptor (7, 8), and E_2 receptor (9).

A common gene in cancer research that also has associated SNPs, p53, recently has received attention for playing an important role in reproduction (10). The set of functions the p53 gene regulates through a multitarget transcription factor model includes tumor suppression, repair of DNA damage, metabolic pathways, regulation of oxidative stress, invasion and motility, cellular senescence, angiogenesis, differentiation, and bone remodeling (11). In reproduction, one of the transcriptional targets of p53 is leukemia inhibitory factor, a cytokine necessary for implantation (10). Although other targets of p53, such as angiogenesis, may play important roles in the success of an IVF cycle, there have been no studies of these relationships yet.

Examining the effects of the p53 SNP on IVF cycle success is important because a change in the p53 codon 72 from Arg to Pro, a single guanine (G) to cytosine (C) mutation, has effects on both the apoptotic and transcriptional functions of

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p53 (12). The Arg (guanine) variant leads to faster apoptotic kinetics, whereas the Pro (cytosine) variant is a better inducer of transcription. The Arg variant is the major allele, whereas the Pro variant is the minor allele. The frequencies of the Arg and Pro alleles are approximately 65% to 70% and 30% to 35%, respectively (13, 14). Because the different aspects of pregnancy require a vast amount of cell turnover, it is reasonable to assume that a gene that affects apoptosis also should affect pregnancy. Additionally, the effect of p53 on the transcription of leukemia inhibitory factor, coupled with the altered transcriptional properties of the codon 72 SNP, further supports investigating the impact of this common SNP on pregnancy. The studies of the same p53 SNP with respect to the incidence of endometriosis, which also is implicated in infertility, have been controversial (15–17).

Prior studies of p53 polymorphisms affecting recurrent implantation failure (18) and recurrent pregnancy loss (19, 20) either lacked statistically significant results or disagreed about p53's effects, which could be attributed to their small sample sizes of approximately 200 patients. The control groups in these past studies were women without infertility. Therefore, the results of these studies are less applicable when counseling patients undergoing infertility treatment because the control populations were fertile populations.

This study attempts to obtain sufficient power by increasing the number of patients and IVF cycles available for analysis and by examining implantation at a per embryo basis for finer granularity. It also attempts to make the results more applicable to the infertility specialist's practice by limiting the conclusions to comparisons made within the patient population seeking IVF for treatment of infertility.

MATERIALS AND METHODS

Study Design and Population

We prospectively enrolled 1,056 female study patients undergoing their first fresh nondonor IVF cycle from the Reproductive Medicine Associates of New Jersey patient population from January 2006 to July 2007. Because no exclusion criteria were set, all patients who chose to participate in the study and who were undergoing their first fresh IVF cycle were included in this study. If the patient's first cycle did not result in at least one embryo successfully implanting, by our definition, they were included in our analysis of subsequent IVF cycles. The analysis continued until either the patient had at least one successful embryo implantation event or the patient discontinued further IVF cycles for any reason. Patients provided follicular fluid or whole blood for DNA extraction before initiating IVF cycles to maintain a prospective study design. The Coast Independent Review Board (Colorado Springs, Colorado) approved this study. All subjects consented to the study before collection of bodily fluids for DNA extraction.

We assessed the impact of the p53 codon 72 SNP on the rates of chemical pregnancy, clinical pregnancy, embryo implantation, live birth, and pregnancy loss. For each of these outcomes, the patients with successful outcomes were compared with those with unsuccessful outcomes to determine whether the p53 codon 72 SNP could explain the differences. A serum hCG level >5 mIU/mL at 14 days after oocyte retrieval defined a successful chemical pregnancy. Evidence of at least one gestational sac by transvaginal ultrasound examination at 5 weeks gestational age defined a successful clinical pregnancy. The number of gestational sacs observed during the clinical pregnancy evaluation defined the number of successful embryo implantations. We obtained the live birth data by patient-physician correspondence or follow-up phone calls to the patients as necessary. Subsequently, loss rates were calculated both for patients who had chemical pregnancies and for those who had clinical pregnancies that did not result in live births.

Genotyping

Deoxyribonucleic acid was isolated from follicular fluid or whole blood following the manufacturer's recommendations with use of the QIAmp DNA Blood BioRobot MDx Kit (Qiagen, Valencia, CA) on an epMotion 5075 VAC automated liquid handling instrument (Eppendorf, Westbury, NY). A NanoDrop 8000 spectrophotometer (NanoDrop Inc., Wilmington, DE) was used to quantify the DNA. In addition, agarose gel electrophoresis was used to characterize the integrity and size of each genomic DNA sample (1% E-Gel; Invitrogen, Carlsbad, CA). Polymerase chain reaction was performed for each DNA sample in duplicate with a TaqMan SNP Genotyping Assay (assay ID = C___2403545_10, dbSNP ID = rs1042522; Applied Biosystems Inc., Foster City, CA) and a GeneAmp 9700 Thermalcycler (Applied Biosystems Inc.) following the manufacturer's instructions. The genotype of each patient's DNA sample was determined with use of the allelic discrimination function within the SDS 2.3 analysis software (Applied Biosystems Inc.) running on a 7900HT Fast Real-Time PCR System (Applied Biosystems Inc.). The fluorescence quantified by the 7900HT was used only for input to the allelic discrimination software and thus will not be reported in this article.

Statistical Analysis

We determined the differences between groups using Fisher's exact test (two-sided) for frequency data, the log-rank test for the difference between survival (life table) curves, and the Kruskal-Wallis nonparametric one-way analysis of variance (ANOVA) test for determining differences between groups with respect to nominal variables. MYSTAT version 12.02.00 (SYSTAT, Chicago, IL) and "R" version 2.6.2 (http://www.r-project.org/) were the statistical software packages used for performing all statistical calculations. The definition of statistical significance was $\alpha < .05$.

RESULTS

There were 1,056, 289, and 72 patients in cycles 1, 2, and 3, respectively, and there were 2,600, 838, and 246 embryos in cycles 1, 2, and 3, respectively. The small number of patients