Negative effect of P72 polymorphism on p53 gene in IVF outcome in patients with repeated implantation failure and pregnancy loss

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Negative effect of P72 polymorphism on p53 gene in IVF outcome in patients with repeated implantation failure and pregnancy loss

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Abstract
Purpose Investigate whether R72P on p53 gene polymorphism has a higher prevalence among women with a history of recurrent implantation failure (RIF) and pregnancy loss (RPL) and its influence in their IVF cycle outcome.
Material and methods p53 polymorphism R72P has been studied in 181 women. The control group included 83 oocyte donors. In the study group 98 women were included: 44 with RIF and 54 with RPL. From the study group, 76 patients underwent IVF-cycles (55 RPL and 21 RIF).
Results The frequency of PP genotypes on p53 among RIF was 11.4 % compared with 18.5 % for RPL and 6 % in controls ($p < 0.01$). There were no significant differences with respect to patient characteristics. Significant differences were reported in pregnancy rate (69.4 % for RR/RP and 33.3 % for PP; $p < 0.05$), embryo implantation rate (33.3 % for RR/RP and 7.3 % for PP; $p < 0.05$) and ongoing pregnancy rate (53.1 % for RR/RP and 14.3 % for PP; $p < 0.05$) among RIF and RPL.
Conclusions This investigation reveals that in RIF and RPL patients R72P on p53 gene is more prevalent than fertile population. Moreover, patients carrying a PP genotype on p53 codon 72 will have less chance to achieve an ongoing pregnancy. This information together with some additional markers will allow development of diagnostic tests for detects risk for RIF and RPL before infertility treatment is initiated.

Keywords p53 polymorphism · RIF, recurrent implantation failure · RPL, recurrent pregnancy loss · P72R

Capsule In RIF and RPL patients P72P on p53 gene i will have less chance to achieve an ongoing pregnancy.

B. Lledo (✉) · A. Turienzo · J. A. Ortiz · R. Morales · R. Bernabeu
Instituto Bernabeu Biotech, Avda. Albufereta, 31, 03016 Alicante, Spain
e-mail: blledo@institutobernabeu.com

J. Ten · J. Llácer · R. Bernabeu
Instituto Bernabeu of Fertility and Gynecology, 03016 Alicante, Spain

Introduction

Recurrent pregnancy loss (RPL) and implantation failure (RIF) are the most common causes of lack of unsuccessful pregnancy after IVF. Underlying causes such as genetic, endocrine, anatomical or autoimmune were found in more than 50 % couples [1]. Several factors such as a variety of environmental as well as lifestyle factors have been considered for idiopathic pathogenesis. Moreover, gene polymorphism may predispose to an increased risk and less attention has been paid to gene polymorphisms [2].

Implantation involves the ability of the implanting blastocyst to invade into the endometrium and to establish its own supply. Suitable trophoblastic invasion results from the balanced effects of growth factors and apoptosis [3, 4]. A number of proteins involved in trophoblastic invasion and angiogenesis have been identified. One such protein is p53, a tumor suppressor, a potent inducer of apoptosis and angiogenesis [5]. Low expression of apoptosis and angiogenesis related genes have been associated with implantation failure [3]. Moreover, recent studies have demonstrated that p53 regulates female reproduction and blastocyst implantation through leukemia inhibitor factor (LIF) [6].

The p53 tumor suppressor gene contains 11 exons, located on chromosome 17p13. A single-nucleotide polymorphism located at the second position of the codon 72 p53 gene consists in either an ancestral C allele derived to a G allele.

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Presence of the C allele results in a proline in codon 72, and presence of the G allele in an arginine. Significant differences in the codon 72 polymorphic form p53 might affect the biological activity of p53. The R72 variant of p53 protein is markedly more efficient than P72 form in inducing apoptosis, LIF expression and suppressing cellular transformation [7].

Previous studies shown P72 polymorphism is a risk factor for RIF [8]. The aim of this work was investigate whether R72P p53 polymorphism has a higher prevalence among women with RIF and RPL and its influence in their IVF cycle outcome.

Material and methods

Study population

RIF was defined as a transfer of a cumulative total of four good quality embryos with negative hCG serum levels. RPL was defined as two or more miscarriages. P53 polymorphism R72P has been studied in 181 women. The control group included 83 oocyte donors. In the study group 98 women were included: 44 with RIF and 54 with RPL. From the study group, 76 patients underwent IVF-cycles (55 RPL and 21 RIF) at Instituto Bernabeu. Both male and female partners in couples RPL and RIF were evaluated.

All the subjects included in the study gave their informed consent to collect peripheral blood samples suitable for molecular analysis. This study involved only retrospective analysis of anonymous medical records and was approved by the Instituto Bernabeu Institutional Review Board.

Genotyping

DNA was isolated from peripheral blood lymphocytes according to the manufacturer instructions (Wizard® Genomic DNA Purification Kit, Promega, USA) and stored at 4 °C. Analysis of p53 gene polymorphism at position 72 was determined using the predesigned TaqMan allelic discrimination assays (rs 1042522, Life Tecnologies Corporation, Carlsbad, NM). Real time PCR was performed using the StepOne plus system from Applied Biosystems in accordance with the manufacturer's instructions.

Ovarian stimulation and oocyte retrieval

After following the Spanish Fertility Act requirements, all the patients received a standard controlled ovarian stimulation protocol. Ovarian response was monitored by transvaginal ultrasound and plasma estradiol concentrations. Oocytes were aspirated by transvaginal, ultrasound-guided needle aspiration under sedation. Sperm and oocyte preparation, fertilization, embryo culture and transfer were performed according to IVF laboratory guidelines.

Statistical analysis

Frequencies of R72 and P72 in each population were calculated. Differences in genotypes distribution were evaluated using chi-square analysis.

Values are presented as averages ± SD for continuous data and percentages for categorical variables. Mann–Whitney test was used for number of retrieved and fertilized oocytes. In order to evaluate possible confounding factors T-student test was performed for continuous data and Fisher's test for categorical data. Logistic regression using number of transferred embryo as confounding factor was performed for pregnancy rate, biochemical pregnancy, miscarriage and ongoing pregnancy. Data were analyzed with Statistical Package for the Social Sciences (SPSS) software (version 20.0, SPSS, Inc., Chicago, IL, USA). A $p<0.05$ was considered significant.

Results

Genotypic and allelic frequencies of p53 codon 72 variants among women experiencing RIF, women with RPL and control women are presented in Fig. 1. The frequency of PP genotypes on p53 gene among study group was 13.6 % vs 6 % in the control group ($p<0.01$) (Fig. 1a). The frequency of PP genotypes on p53 gene among women experiencing RIF was 11.4 % vs 18.5 % for those with RPL and 6 % in controls ($p<0.01$) (Fig. 1b). RP genotypes occurred in 47.7 % of women with RIF, in 29.6 % of RPL and 28.9 % of control women. RR genotypes were 40.9 % among women experiencing RIF, 51.9 % women with RPL and 61.5 % controls.

The results of the association between the p53 codon 72 polymorphism and recurrent implantation failure and pregnancy loss were analyzed according to recessive model (PP vs PR/RR) as previous published data [8]. There were no significant differences with respect to patient age, follicular phase length, endometrial thickness, oocyte retrieved, MII oocyte, number of fertilized oocytes and total embryos (Table 1). Difference in the number of transferred embryos between the genotypes was reported (Table 1). To avoid confounding effects, we adjusted the statistical analysis using the number of transferred embryos.

The impact of p53 P72 on implantation and pregnancy after IVF was investigated in patients with RIF and PRL. P72 appears to be a risk factor for these patients. The embryo implantation rate was significantly lower in patients homozygous P72 (33.3 % for RR/RP and 7.3 % for PP; $p=0.001$), which leads to a significantly lower pregnancy rate (69.4 %

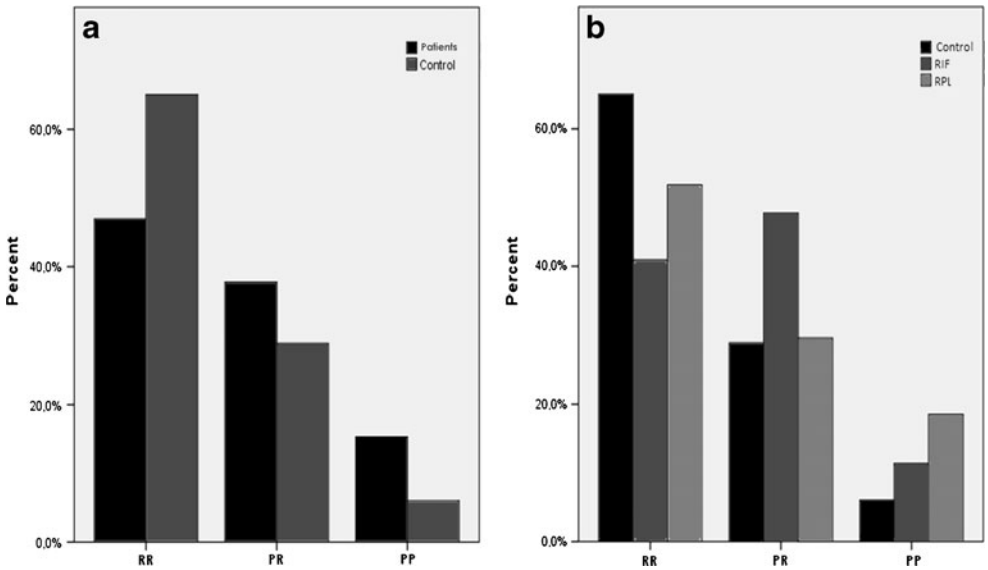

Fig. 1 Frequency comparison of P72 between different populations: **a** on the control and study group **b** on the RIF and RPL groups

for RR/RP and 33.3 % for PP; $p=0.011$). The ongoing pregnancy rate (53.1 % for RR/RP and 14.3 % for PP; $p=0.003$) was lower among women carrying PP genotype (Table 1). Although no statistically difference was showed in miscarriage rate a tendency was reported (12.0 % for RR/RP and 33.3 % for PP; $p=0.247$). The limited number of pregnancy achieved by the PP group could be the main cause to show a statistical difference. The P72 polymorphism on p53 gene continues correlating with the main cycle outcome measures when study group was divided in RIF and RPL ($p<0.05$).

Table 1 Description of patient group and IVF results

	RR/RP ($n=55$)	PP ($n=21$)	*P*
Female age (y)	37.7±3.7	37.9±3.3	0.79
Male age (y)	35.4±3.7	37.3±3.2	0.20
Follicular phase length (days)	18.5±4.3	19.8±3.3	0.318
Endometrial thickness (mm)	8.1±1.2	8.6±1.6	0.476
N° of oocyte retrieved	11.8±12.6	12.7±5.4	0.150
MII oocyte	8.0±4.9	8.2±4.9	0.345
N° of fertilized oocytes	6.6±4.1	5.6±6.0	0.345
Total embryo	6.2±3.4	5.6±2.0	0.465
Total Embryo transferred	2.2±0.4	2.0±0.4	0.026
Implantation rate (%)	33.3	7.3	0.001
Pregnancy rate (%)	69.4	33.3	0.001*
Biochemicl pregnancy rate (%)	16.3	19.0	0.389*
Miscarriage rate (%)	12.0	33.0	0.247*
Ongoing pregnancy rate (%)	53.1	14.3	0.015

Mann–Whitney test was used for number of retrieved and fertilized oocytes

T-student test was performed for continuous data

Fisher's test was performed for categorical data

*p-value from the logistic regression adjusted with N° of transferred embryos

Discussion

We investigate polymorphism of the p53 suppressor gene that encodes either R or P at position 72 in order to gain further insight into their relationships with RIF and RPL after IVF. To the best of our knowledge, these data show for the first time significant differences in allelic frequencies and IVF outcome in women experiencing RPL and RIF were compared with controls. Our data show women with the PP genotype had a higher risk for lower pregnancy rate [OR = 4.32, 95 % confidence interval CI = 1.39–13.51] and ongoing pregnancy rate [OR = 8.85, 95 % CI = 2.13–37.04] compared with the RR/RP genotypes.

Successful pregnancy depends on the ability of the embryo to achieve appropriate extent to trophoblastic proliferation, enzyme digestion and invasion into endometrium as well as, once implanted, to induce its own blood supply by adequate angiogenesis [9, 10]. Although of these complex mechanisms are not well understood, it is clear that multiple signals are needed to synchronize blastocyst maturation and uterine receptivity. Alterations in one or more of these factors can lead to implantation failure. Once implanted, the embryo continues to survive and grow by stimulating its own blood supply. Consequently, an efficient cross-talk between embryo and mother is necessary. Therefore, abnormal angiogenesis and/or apoptosis either in cytotrophoblasts and/or blood vessels can result in imbalances of cell differentiation leading to miscarriage [11].

Although many implantation failures and pregnancy losses involves chromosomal abnormalities, there is often no

apparent cause [12]. The results of the present study add to the data supporting that genetic factors other than chromosomal abnormalities contribute to elucidated unexplained causes of RIF and RPL.

P53 codon P72 induces high levels of G1 cell cycle arrest than R72, thus decreasing proliferation [13]. Imbalances in the regulated processes of cellular proliferation or differentiation caused by an increased number of cells arrested at the G1 chechkpoint, as may occur in the p53 P72 variants compared with the p53 R72 variants, might cause inadequate trophoblastic growth and then implantation failure. The lower level of apoptosis in p53 P72 allele carriers might cause misguided growth of cells or tissues leading to miscarriage [14].

Moreover, p53 plays a significant role in endometrial receptivity through the regulation of Leukaemia inhibitory factor (LIF). LIF is the cytokine produced and secreted by the endometrium, regulating implantation [6]. Sufficient uterine LIF protein is an essential condition for implantation. R72 was more active than p53 P72 in transactivating LIF and then P72 result in the reduced uterine LIF levels and decreased implantation rates in women [15].

This investigation reveals that in RIF and RPL patients P72 on p53 gene is more prevalent than fertile population. Our results support previous studies indicating P72 is involved in human fertility [11], especially at first stages [16]. The frequency of PP genotypes was found significantly higher among the women with RPL than the other groups according to the meta-analysis published by Tang in 2011 [8]. Moreover, our data shows patients carrying a PP genotype on p53 will have less chance to achieve an ongoing pregnancy according to results published by Kang et al. 2009 [15] including couples with unexplained infertility.

Genotyping p53 gene is an important factor for determining the prognosis of IVF cycles on RIF and RPL women because P72 on p53 gene is more prevalent in RIF and RPL patients than fertile population and it is predictive in the IVF cycle. This information together with some additional markers will allow developments of diagnostic tests for detect risk for RIF and RPL before infertility treatment is initiated.

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