Association of progesterone receptor polymorphisms with recurrent implantation failure after in vitro fertilization and embryo transfer

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Received: 14 September 2007 / Accepted: 28 February 2008 / Published online: 5 April 2008
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Abstract

Introduction Progesterone is the hormone of pregnancy and is required for its initiation. The actions of progesterone are mediated by the progesterone receptor. Polymorphic variants of human progesterone receptor genes have been implicated in implantation failure.

Materials and methods We, therefore, investigated the prevalence of H770H(C/T genotype), V660L polymorphism and a 306 bp Alu insertion in exon 7 of the progesterone receptor among women with history of recurrent implantation failure to determine whether any of these polymorphisms may serve as a risk factor for implantation failure. DNA was extracted from the buccal swabs obtained from 66 women experiencing implantation failure and 75 fertile control women. PCR amplification of fragments was purified and the DNA sequenced to identify the polymorphism. The frequencies for the three variants were 27% for H770H, 21%

for V660L and 0% for the 306 bp Alu insertion in exon 7 among women with implantation failure compared with control women of 25% for H770H and 24% for V660L and 0% for the 306 bp Alu insertion in exon 7.

Discussion No significant differences in the overall allelic frequency of progesterone receptor variants was seen when women experiencing recurrent implantation failure were compared with control women.

Conclusion We conclude that the H770H and V660L and PROGINS progesterone receptor polymorphisms are not markers that can identify women at risk for recurrent implantation after IVF/ET.

Keywords Progesterone · Receptor polymorphisms · Recurrent implantation failure · RIF

Capsule H770H, V660L and PROGINS progesterone receptor polymorphisms were not found to be associated with recurrent implantation failure after IVF/ET.

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Introduction

Progesterone is the hormone of pregnancy. It is required in all mammals for development and maintenance of an endometrium that allows implantation and supports pregnancy. Progesterone deficiency or luteal phase defects have been proposed as an etiology of implantation failure [1]. Implantation failure is the most frequent cause of lack of pregnancy after in vitro fertilization (IVF) and embryo transfer (ET) at a time when progesterone is being administered exogenously [2]. The actions of progesterone are mediated by the progesterone receptor. Progesterone and progesterone receptor have particular importance in permitting implantation. Polymorphic variants of human progesterone receptor genes have been described and implicated in implantation failure [3, 4]. In 1995, Rowe et al. [5] described a small (306 bp) insertion in intron G of the T2 allele of the human progesterone receptor and named

it “PROGINS”. Subsequently, two-point mutations were found to be linked to the PROGINS mutation Kieback et al. [6]. These two mutations included a G to T substitution in exon 4, causing a Valine to Leucine change (V660L) and a C to T substitution in exon 5 (H770H). These three variants of the progesterone receptor have been referred to in the literature as PROGINS complex. One study demonstrated a significant increase in PROGINS complex mutations among women with unexplained infertility compared with fertile women [3] while another investigation reported the likelihood of a woman carrying the V660L progesterone receptor polymorphism increases with the number of failed attempts at implantation after IVF/ET [4]. To determine which of the variants of the PROGINS complex are associated with implantation failure, we undertook the present study to compare the prevalence of polymorphic variants of H770H(C/T) genotype, V660L polymorphism and the 306 bp Alu insertion of the human progesterone receptor gene among women experiencing recurrent implantation failure after IVF/ET with fertile control women.

Materials and methods

Patients

A total of 66 women with a history recurrent implantation failure after IVF/ET and 75 fertile women with at least two live births were included in the study. Recurrent implantation failure was defined as a cumulative of 8 cleaved embryos transferred or four blastocysts transferred without a successful clinical pregnancy characterized by either a negative pregnancy test 2 weeks after embryo transfer or a chemical pregnancy loss [7]. All transferred embryos were judged by the embryologist to be of good quality. A preclinical or chemical pregnancy losses were defined as two rising human chorionic gonadotropin (hCG) serum concentrations >2 mIU/ml and no intrauterine sac visible on ultrasonographic examination at 6 weeks of gestation [7]. Both male and female partners of couples experiencing recurrent implantation failure were evaluated. All women were examined by ultrasonography or hysterosalpingogram for detection of anatomic abnormalities of the genital tract and had blood drawn for testing for immunologic risk factors including antiphospholipid antibodies. Semen analysis and Sperm DNA Integrity assay were performed with normal results on all male partners. Records of previous IVF/ET cycles were reviewed.

Seventy-five (75) fertile women with at least two live births and not more than one abortion provided that the last pregnancy was a normal delivery served as controls. Buccal swabs were obtained from all women and analyzed for

H770H (C/T genotype), V660L polymorphism and the 306 bp Alu insertion in exon 7.

Progesterone receptor analyses

DNA was extracted from buccal swabs using a Qiagen QIAamp DNA mini kit (catalog no. 51306, Qiagen, USA) according to the manufacturer's instructions. Polymerase chain reaction (PCR) was then conducted using established protocols, summarized as follows:

Primers for PCR of PROGINS were (F) 5'-GTCGTAACTTTCGTCTTCCAGCA-3', (R) 5'-TCGCCCTGGTCCCAAGGAAGATT-3'; for V660L (F) 5'-GAGTTTATATATTTACATGA-3', (R) 5'-ATAGATCACATCTGGTCAATG-3'; and for H770H (F) 5'-CTACGCATAC CTATTTTCAGAT-3', (R) 5'-CTCCAAATATAAAATTACCGTA-3'. PCR reactions were 50 µl total volumes, with 0.8 µM final concentration for each primer. PCR buffer and Taq Polymerase were from New England Biolabs (NEB). Thermal cycling was conducted using a Perkin-Elmer GeneAmp 2400 thermal cycler. Annealing temperatures were 58.0°C for PROGINS; 48.0°C for V660L; and 49.0°C for H770H. The thermal cycling protocol was 95°C/2 min, followed by 35 cycles of [95°C/1 min → T°C/30 s → 72°C/1 min]; then 72°C/5 min and 4°C/hold. (T* is the annealing temperature.) PCR products were visualized with ultraviolet light using 3.0% agarose gel electrophoresis (with ethidium bromide). The fragment sizes were: PROGINS 200 bp; V660L 240 bp; and H770H 370 bp. For DNA sequencing, PCR products were purified using either the Qiagen QIAquick PCR Purification Kit (catalog no. 28104). The purified PCR products were DNA sequenced using the Applied Biosystems BigDye Terminator method on an ABI Prism 3100 genetic analyzer.

Statistical analysis

Sample size was calculated using the Pisarska et al. [3] data of PROGINS and H770H polymorphisms prevalence of 42% for patients experiencing unexplained infertility and 14% for controls. A sample size of 136 would be required for an alpha error of 5% and a statistical power of 90% to confirm their results. With a total of 142 patients included in the current study, power analysis reveals that the probability is 90% that this study will detect a relationship between the polymorphisms and implantation failure at a two sided 5% significance level, if the true difference is 28% as indication by Pisarske et al. [3].

Frequencies of H770H, V660L, and PROGINS among women experiencing recurrent implantation failure were compared with fertile control women, and the differences were analyzed by one-way analysis of variance (ANOVA). Significant differences were defined as $p < 0.05$.

Results

The characteristics of women experiencing recurrent implantation failure and control women are depicted in Table 1. The number of previous IVF cycles among women experiencing implantation failure after IVF/ET was 4.3. All women were less than 40 years of age.

No mutations in the 306 bp Alu insertion in exon 7 were observed in either patient experiencing recurrent implantation failure or control women. Polymorphic frequencies of progesterone receptor variants in H770H and V660L among patients experiencing recurrent implantation failure and control women are presented in Table 2. The frequencies for the variants were 27% for H770H, 25% for V660L among women with implantation failure compared with control women of 25% for H770H and 24% for V660L. No significant differences in the overall allelic frequency of progesterone receptor variants was seen when women experiencing recurrent implantation failure were compared with control women.

Discussion

We investigated mutations of the progesterone receptor gene to seek further insight into their relationships with recurrent implantation failure after IVF/ET. No significant differences in genotypic frequencies were found when women experiencing recurrent implantation failure were compared with controls. None of the cases or controls displayed the 306 bp Alu insertion in exon 7. These results are not in agreement with previous reports of associations between progesterone receptor mutations and adverse reproductive outcomes including unexplained infertility [3], implantation failure after IVF/ET [4] and unexplained recurrent spontaneous abortions [8]. Pisarska et al. [3] found an increase in the prevalence of PROGINS mutation measured as 306 bp Alu insertion and H770H polymorphisms among 26 women with the diagnosis of unexplained infertility compared with 28 control women (42% vs. 14%). All of their mutations were heterozygous. In the current study, there were two women in the control group who had homozygous mutations (one H770H and one for

Table 2 Frequency of progesterone receptor mutations among women experiencing recurrent implantation failure after in vitro fertilization and embryo transfer compared with fertile control women

Progesterone receptor mutations	RIF (<i>n</i> =66)		Controls (<i>n</i> =75)		<i>p</i> Value
	<i>N</i>	Percent	<i>N</i>	Percent	
H770H					
Heterozygous	16	24.2	18	24.0	NS
Homozygous	2	3.0	1	1.3	NS
Total	18	27.3	19	25.3	NS
V660L					
Heterozygous	14	21.2	17	22.7	NS
Homozygous	1	1.5	1	1.3	NS
Total	15	22.7	18	24.0	NS
Total alleles (<i>n</i> =264)	36	20.8	39	14.8	NS

V660L), yet they had two successful pregnancies. This observation suggests that homozygous mutations are not sufficient to prevent implantation and adds further support for the lack of association of these polymorphisms and implantation failure. None of the 66 women with a history of recurrent implantation failure nor any of the 75 fertile control women in the present study displayed the 306 bp Alu insertions. Cramer et al. [4] found that women possessing the H770H T allele had a decreased risk for implantation failure after IVF/ET, but only among women less than 35 years of age. Moreover, among women with a H770H C/C genotype, there was an increase in the proportion of +331 G/A carriers as the number of implantation failures increased from 8.6% of women with no failed attempts to 40% among women with five or more embryo transfers with no successful pregnancies [4]. The significant trend ($p=0.03$) for implantation failure with the combine progesterone receptor polymorphisms suggests a cumulative effect of the mutations. Among the 19 women experiencing recurrent implantation failure that displayed progesterone receptor polymorphisms, 14% or 74% had both H770H and V660L and 33 of the five with only one of the polymorphisms were homozygous mutations adding further support for the additive effects of mutations rather than an association with a specific mutation. A similar observation of additive effects of multiple gene mutations on implantation failure was reported with the thrombophilia genes [9]. However, in the current study the control women also displayed multiple rather than single gene polymorphisms suggesting disequilibrium of inheritance. An association between progesterone receptor polymorphisms and unexplained recurrent miscarriage was both reported [8] and refuted [10]. In addition to the fact that the observations could not be repeated and therefore considered as invalid, three other explanations for the discrepant results are (1) The etiologies for recurrent miscarriages are

Table 1 Characteristics of women with recurrent implantation failure (RIF) and control women

	RIF <i>n</i> =66	Controls <i>n</i> =75
Age (years)	35.1	37.5
No. gravid (%)	23 (35%)	32 (100%)
No. parous	10 (15%)	32 (100%)
No. abnormal immunologic tests (%)	5 (8)	

multifactorial with the major cause being attributed to chromosomal abnormalities of the embryo [11]. The relative contribution of progesterone receptor defects would require very large sample sizes to detect and effect. (2) Since recurrent miscarriages usually occur after 6 weeks of gestation, implantation has already occurred and the effect of progesterone receptor minimal compared with earlier during the process of implantation [12]. (3) The study that showed an association between progesterone receptors polymorphisms and recurrent abortions evaluated 3 single nucleotide polymorphisms including S334T, V660L and H770H whereas the report of no association between progesterone receptor gene polymorphisms and idiopathic recurrent miscarriage examined the PROGIN 306 bp insertion polymorphism in intron G only [10].

The initiation of pregnancy requires specifically timed development of the endometrium to receive the implanting blastocyst. Accurate assessment of the adequacy of the receptive uterus at the molecular level would be helpful in developing strategies to improve the pregnancy success rate of existing assisted reproductive technologies. By comprehensively delineating the molecular correlates of the receptive endometrium clinical predictors of the receptively window can be identified. Recent reports have suggested that causes of recurrent implantation failure include over or under expression of genes that encode for proteins necessary for successful implantation [13, 14]. Since progesterone is essential for the development of a receptive endometrium, it seemed reasonable to look at progesterone receptor gene defects among women experiencing recurrent implantation failure. However, one could argue that women with a history of recurrent implantation failure comprise a heterogeneous group, thus comparison of rates of progesterone receptor mutations with fertile women might not provide a reliable source of information. The results of the current investigation do not confirm previous studies [3, 4] that the polymorphisms of H770H, V660L and PROGINs are associated with reproductive failure. It is hoped that identification of other key molecular signals essential for the establishment of a receptive uterus will provide opportunities to develop new approaches to correct implantation failure.

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