Association of estrogen and progesterone receptor polymorphisms with idiopathic thin endometrium

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The research question is as follows: Are estrogen and progesterone receptor genotypes associated with thin endometrium? We performed a prospective cohort study of 129 patients who underwent preimplantation genetic testing for aneuploidies. These patients were categorized according to endometrial thickness: >7 mm control group ($n = 94$) and ≤7 mm study group ($n = 35$). Polymorphisms in the genes *ESR1* (rs9340799 and rs3138774), *ESR2* (rs1256049 and rs4986938), and *PGR* (rs1042838) were analyzed. Regarding genotype distribution, the GA/AA genotype frequency for rs4986938-*ESR2* was higher in the thin endometrium group (80% in the study group vs. 50% in the control group; $P = 0.002$), as well as the GG genotype of *PGR* (8.6% in the study group vs. 0% in the control group; $P = 0.002$). No differences were observed for the remaining genotypes. In terms of clinical data, the pregnancy rate after euploid embryo transfer was lower in patients with the AA genotype for rs4986938-*ESR2* (18.2% AA vs. 40.8% GA vs. 44.0% GG; $P = 0.039$). Finally, a predictive pregnancy model was developed using clinical data and *ESR2* and *PGR* genotypes, with an area under the curve of 0.76, sensitivity of 64%, and specificity of 76%. The genetic variants rs4986938 in the *ESR2* gene and rs1042838 in the *PGR* gene seem to correlate with idiopathic thin endometrium. In addition, the rs4986938 polymorphism in the *ESR2* gene is associated with pregnancy rate. Finally, a predictive model combining clinical data and patient genetic profiles has been proposed to predict clinical pregnancy outcomes.

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Introduction

Embryo implantation is a crucial process in reproduction, requiring effective communication between the embryo and the endometrium for a successful pregnancy. Endometrial thickness (EMT) has emerged as a critical factor for endometrial receptivity. Studies have shown that patients with an EMT of less than 8 mm have a lower chance of achieving pregnancy, in both fresh and frozen-thawed embryo transfer cycles [1]. There is currently no consensus on the definition of thin endometrium, with thresholds of 6, 7, or 8 mm reported in the literature. Recent research suggests that an EMT below 6 mm significantly reduces the likelihood of pregnancy, with a minimum thickness of 7 mm recommended for adequate receptivity [2]. However, some patients present with idiopathic thin endometrium, where the endometrial lining remains persistently thin despite adequate estrogen levels. The management of thin endometrium is a common challenge in assisted reproduction.

Polymorphic variations in genes involved in sex hormone signaling, particularly in the estrogen receptors (*ESR1* and *ESR2*) and progesterone receptors (*PGRs*), have been suggested as risk factors for pregnancy loss [3]. The *ESR1* and *ESR2* genes are highly polymorphic with the majority of variants located in intronic regions. The *PGR* gene, located on chromosome 11, is also highly polymorphic, with several variants associated with reproductive complications.

The aim of this study was to investigate the association of estrogen receptors (*ESR1* and *ESR2*) and *PGR* polymorphisms with idiopathic thin endometrium and their effect on pregnancy rates in cycles after euploid embryo transfers.

Materials and methods

This observational study included a total of 129 patients undergoing preimplantation genetic testing for aneuploidies (PGT-As) at Instituto Bernabeu between February 2018 and January 2023. Participants were divided into two groups according to the EMT: a study group consisting of 35 individuals with EMT less than or equal to 7 mm and a control group consisting of 94 individuals with an EMT greater than 7 mm. Exclusion criteria included any history of significant uterine abnormalities, abnormal hysteroscopy, or abnormal hormone levels.

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The study was approved by the Instituto Bernabeu Review Board, and all participants gave informed consent before enrollment.

All participants underwent standard in vitro fertilization (IVF) PGT-A procedures. Genetic analysis was performed using Veriseq (Illumina, San Diego, California, USA). Embryos were vitrified and transferred after analysis.

Endometrial preparation protocols were standardized and included both natural and artificial methods. The choice of preparation method was at the discretion of the treating physician and based on individual patient profiles.

For genotyping rs1256049, rs9340799, rs4986938, and rs1042838 of the *ESR1*, *ESR2*, and *PGR* genes, DNA was isolated from peripheral blood using the commercial MagMAX DNA Multi-Sample Ultra 2.0 kit (Thermo Fisher Scientific, Thermo Fisher, Madrid, Spain) and the KingFisher Duo Prime system (Thermo Fisher Scientific). Polymorphism analysis was performed using predesigned TaqMan Allelic Discrimination Assays (Life Technologies, Madrid, Spain). Real-time PCR was performed using StepOnePlus (Thermo Fisher Scientific).

Statistical analysis was performed using [SPSS 23.0, R 4.3.1]. Descriptive statistics were calculated for all variables, and differences between groups were assessed using appropriate tests. A *P*-value of <0.05 was considered statistically significant.

For risk assessment, odds ratios (ORs) were calculated for the association between genotypes and EMT, along with 95% confidence intervals (CIs) including endometrial preparation as confounding factor.

In addition, a predictive model for pregnancy outcome was developed using clinical data and genotypes from the *ESR2* and *PGR* genes. The predictive model was developed using multivariate logistic regression. To achieve a parsimonious model, variables included in the final model were selected based on minimizing the Akaike Information Criterion. This metric evaluates the model's goodness of fit while accounting for its complexity, prioritizing simpler models with strong predictive performance.

Results

A total of 129 participants were included in the study, with 35 individuals in the study group and 94 in the control group. Patient characteristics, clinical data, and genotype frequencies are shown in Table 1. The mean age of females in the study group was significantly younger than in the control group (32.80 vs. 35.63 years; $P = 0.021$). No significant differences were observed in male age, weight, height, or BMI.

As expected, there was a significant difference in EMT, which was notably lower in the study group (6.57 mm) compared with the control group (8.97 mm). The number of oocytes and metaphase II retrieved, embryo quality,

Table 1 Patient's demographic characteristics and IVF data

	Total ($n = 129$)		Study group ($n = 35$)		Control group ($n = 94$)		
	Mean	SD	Mean	SD	Mean	SD	*P*
Female age (years)	34.86	6.25	32.80	6.43	35.63	6.03	0.02[a]
Male age (years)	39.22	7.19	38.09	7.34	39.64	7.13	0.28[a]
Weight (kg)	61.44	11.09	62.21	10.57	61.16	11.34	0.39[a]
Height (m)	1.65	0.06	1.64	0.05	1.65	0.07	0.49[b]
BMI (kg/m^2)	22.60	3.28	2.08	3.41	22.42	3.24	0.28[a]
Endometrium thickness (mm)	**8.32**	**1.56**	**6.57**	**0.79**	**8.97**	**1.24**	**<0.001**[a]
Progesterone (ng/ml)	20.94	8.90	18.91	11.07	21.51	8.19	0.08
Retrieved oocytes	11.00	5.55	11.00	5.47	11.00	5.61	0.85[a]
Retrieved MII	9.47	4.51	9.74	4.33	9.37	4.60	0.67[a]
Embryo biopsied	4.75	2.41	4.31	2.07	4.91	2.51	0.25[a]
Endometrium preparation							
Artificial (%)	44.70		62.90		39.80		<0.05[c]
Day of embryo transfer							
D + 5 (%)	69.80		74.30		68.10		0.60[d]
Embryo quality							
A (%)	59.70		68.60		56.40		0.22[d]
B (%)	38.80		28.60		42.60		
IVF outcome							
Positive beta-HCG (%)	50.40		54.80		47.80		0.50[c]
Biochemical miscarriage (%)	10.00		9.70		10.10		0.99[d]
Clinical pregnancy (%)	40.00		35.50		41.60		0.55[c]
Clinical miscarriage (%)	14.60		27.30		10.80		0.33[d]
Ongoing Pregnancy (%)	34.20		25.80		37.10		0.25[c]

Bold values are referenced to *P* values lower than 0.01.
HCG, human chorionic gonadotropin; IVF, in vitro fertilization; MII, metaphase II.
[a]Wilcoxon rank sum test.
[b]Welch two sample *t*-test.
[c]Pearson's chi-square test.
[d]Fisher's exact test.

Table 2 Genotype frequency

Genotype frequency (%)	Total ($n = 129$)	Study group ($n = 35$)	Control group ($n = 94$)	OR (95% CI) (univariable)	OR (95% CI) (multivariable)
rs1256049					
GG	88.4 (114)	85.7 (30)	89.4 (84)	1.40 (0.41–4.29, $P = 0.567$	1.98 (0.44–8.97, $P = 0.367$)
GA	11.6 (15)	14.3 (5)	10.6 (10)		
rs9340799					
AA	39.5 (51)	37.1 (13)	40.4 (38)	1.00 (0.42–2.38, $P = 0.994$)	1.12 (0.39–3.27, $P = 0.831$)
AG	45.7 (59)	42.9 (15)	46.8 (44)		
GG	14.7 (19)	20.0 (7)	12.8 (12)		
rs4986938					
GG	41.9 (54)	20.0 (7)	50.0 (47)	**4.00 (1.67–10.76, $P = 0.003$)**	**4.79 (1.75–15.64, $P = 0.004$)**
GA	40.3 (52)	60.0 (21)	33.0 (31)		
AA	17.8 (23)	20.0 (35)	17.0 (94)		
rs1042838					
GG	2.3 (3)	8.6 (3)	0 (0)	**8.62 (1.06–177.80, $P = 0.066$)**	**8.99 (1.01–194.54, $P = 0.048$)**
GT	24.2 (31)	20.0 (7)	25.8 (24)		
TT	73.4 (94)	71.4 (25)	74.2 (69)		

Bold values are referenced to *P* values lower than 0.01.
Logistic regression including confusion factors. GG and AA are related to DNA bases.
A, adenine; C, cytosine; CI, confidence interval; G, guanine; OR, odds ratio; T, thymine.

the number of embryos biopsied, and the day of the biopsy did not show differences between the groups (Table 2).

Regarding endometrial preparation, 44.70% of the total cohort underwent artificial preparation, with a difference between groups (62.90 study group vs. 39.80 control group; $P = 0.023$).

The IVF outcomes indicated that the positive beta-human chorionic gonadotropin rate, biochemical miscarriage rate, clinical pregnancy rates, and clinical miscarriage and ongoing pregnancy rates were not significantly different between groups (Table 1).

Genotype frequencies were assessed for several polymorphisms (Table 2). For rs1256049 and rs9340799, similar frequencies of different genotypes were found in both groups. Notably, for rs4986938, the GG genotype was significantly lower in the study group compared to the control group (20.0 vs. 50.0%; $P = 0.0063$). Patients carrying the A allele had a five-fold higher risk of having a thin endometrium (OR: 4.79, 95% CI: 1.75–15.64, $P = 0.004$). Finally, for rs1042838, the GG genotype was found in 8.6% of the study group and absent in the control group, which was statistically significant ($P = 0.0334$). Patients carrying the G allele have a nine-fold higher risk of having a thin endometrium (8.99, 95% CI: 1.01–194.54, $P = 0.048$).

Finally, we compared IVF outcomes according to the *ESR1*, *ESR2*, and *PGR* genotypes. Our results showed that pregnancy rates were lower in patients with the AA genotype for rs4986938 in *ESR2* gene after euploid embryo transfer (18.2% AA vs. 59.2% GA vs. 56.0% GG; $P = 0.039$). A predictive model for pregnancy was developed using clinical data and ESR2 and PGR genotypes, yielding an area under the curve of 0.76, with a sensitivity of 64% and a specificity of 76% (Fig. 1).

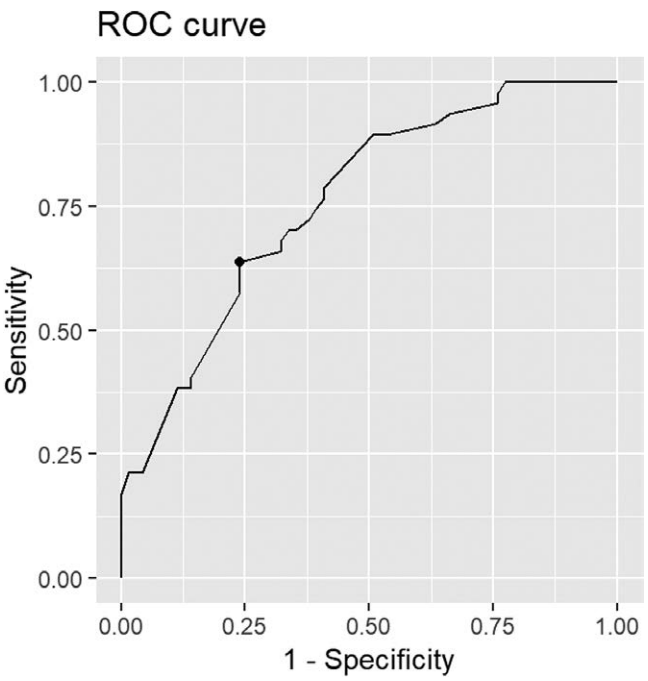
Discussion

This study identified a significant association between the polymorphisms in *ESR2* and *PGR* genes with idiopathic thin endometrium. In addition, the polymorphism in *ESR2* was associated with lower pregnancy rates, highlighting the importance of genetic profiling in endometrial receptivity and reproductive outcomes.

Estrogen contributes to the entire pregnancy by affecting fertility, implantation, embryonic and fetal growth, reproductive cycle, uteroplacental blood flow, and maintenance of gestation. Evidence has identified that progesterone plays a role in pregnancy loss as decreased progesterone in early pregnancy is related to elevated odds, and treatment with progesterone may be beneficial for its prevention. Estrogen and progesterone exert their biological effects by binding to the cytosolic estrogen and *PGRs*, respectively. The evidence suggests that *ESR* gene polymorphisms can influence diverse estrogen-dependent pathways probably affecting the vascular tone and flow, consequently resulting in disruption of pregnancy establishment and maintenance. Also, polymorphic variations on *PGR* gene can lead to functionally resistant receptor. However, little is known about the influence of these polymorphisms and the thin endometrium.

Although our findings are in agreement with existing literature that highlights the role of estrogen receptors in modulating endometrial function and receptivity, our results disagree with the limited evidence suggesting the association of *ESR1* [4] and *ESR2* gene polymorphisms [5]. Nevertheless, these discrepancies could be explained by differences in sample size, genetic background, characteristics of the studied populations, and ethnicity across individual studies. Although the polymorphisms rs4986938-ESR2 do not cause amino acid changes, they may be in linkage disequilibrium with various regulatory sequences, consequently affecting the gene function or expression [6], explaining our findings.

Fig. 1



ROC curve for the predictive model for pregnancy outcome. ROC, receiver operating characteristic.

On the other hand, to the best of our knowledge, this is the first time to investigate the association between *PGR* polymorphisms and thin endometrium. The rs1042838-PGR polymorphism, known as PROGINS polymorphism, reduces the response to progesterone [7] acting as a risk-modulating factor in several gynecological disorders. It is also widely recognized for its influence on achieving a successful pregnancy [8]. Therefore, the progesterone resistance may explain the inability of the endometrium to grow properly, which support our findings.

Finally, the development of a predictive model based on clinical data and studied genotypes emphasizes the potential for integrating genetic information into clinical practice to enhance individualized treatment approaches in assisted reproductive techniques. Further studies using a pharmacogenetic approach are needed.

In conclusion, our results suggest that genetic predisposition may influence both EMT and reproductive outcomes. Early identification of patients who may develop inadequate endometrial growth, along with appropriate therapeutic management, could benefit these patients.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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