Value of autoimmune screening and immunomodulatory treatment to improve subsequent pregnancy outcomes in patients undergoing preimplantation genetic testing

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Abstract

Introduction: We aimed to evaluate the clinical utility of autoimmune screening and the effectiveness of immunomodulatory treatment in preimplantation genetic testing (PGT) patients.

Material and methods: In this retrospective cohort study, 151 PGT patients who experienced a failed first frozen embryo transfer (FET) were divided into two groups. Patients underwent immune marker screening before their second FET. Among them, 42 were classified into the immune-positive group and received targeted interventions, while 109 were categorized into the immune-negative group, which was further divided into an empirical medication subgroup (n = 43) and a no-intervention subgroup (n = 66). Baseline characteristics and pregnancy outcomes were analyzed using statistical methods, including univariate and multivariate logistic regression analyses.

Results: We found that among PGT patients with first transfer failure, 28% (42/151) showed abnormal immune markers. Targeted therapy in immune-positive patients was associated with significantly improved clinical pregnancy rates compared to their first unscreened cycle (64.29% vs. 33.33%, p = 0.005), while reducing the miscarriage rate. In contrast, empirical immunomodulation in immune-negative patients did not provide any additional benefit in terms of live birth rate (39.53% vs. 43.94%, p = 0.649). Multivariate analysis confirmed that embryo quality was an independent positive predictor of both hCG positivity (p = 0.034) and clinical pregnancy (p = 0.022), whereas maternal age showed an inverse association with the likelihood of success (p < 0.05).

Conclusions: Immune abnormalities are relatively prevalent in the PGT population. Targeted immunomodulatory interventions in immune-positive patients were associated with improved pregnancy outcomes. We recommend that these patients should undergo immune screening and targeted interventions before embryo transfer. However, empirical therapy in immune-negative individuals is unjustified.

Key words: preimplantation genetic testing, euploid blastocyst, autoimmune screening, targeted immunomodulation, empirical therapy.

Introduction

Preimplantation genetic testing (PGT) has revolutionized assisted reproductive technology (ART) by enabling the selection of euploid embryos before transfer [1]. This advancement has significantly improved implantation rates and reduced the risk of miscarriage among patients with inherited diseases, recurrent pregnancy loss (RPL), recurrent implantation failure (RIF), or advanced maternal age [2–4]. Notably, despite the increasing adoption of PGT technology, some studies indicated that PGT may not enhance live birth rates in cycles involving the transfer of chromosomally normal embryos, while simultaneously prolonging treatment duration and increasing the financial burden [5]. These observations highlight that embryonic euploidy alone is not sufficient to ensure reproductive success [6]. Critical maternal factors – particularly endometrial receptivity and the dynamic crosstalk between the embryo and endometrium – must be optimized to overcome non-embryonic barriers to implantation [7]. Central to this interplay is the maternal immune system, which plays a pivotal role in maintaining the delicate balance required for the successful establishment of pregnancy [8].

The immune system plays a critical role in normal implantation, maternal-placental-fetal crosstalk and embryonic development. Emerging evidence indicates that maternal immune dysregulation, particularly the presence of autoantibodies, may negatively affect endometrial receptivity, placental development, and the maintenance of early pregnancy [9–11]. Clinical studies have also demonstrated that established autoimmune diseases, such as systemic lupus erythematosus (SLE) and primary Sjögren syndrome (pSS), may negatively impact the outcomes of ART [12, 13].

In recent years, advances in reproductive immunology have increasingly highlighted the pathological significance of 'subclinical immune disorders', including nondiagnostic abnormalities such as antinuclear antibody (ANA) positivity and markedly elevated anti-phospholipid antibodies (aPL) [14–16]. Fundamental studies have revealed that these subtle immune markers alterations can disrupt the embryo-endometrial dialog through multiple mechanisms. Specifically, aPL may reduce endometrial receptivity and disrupt decidualization, affecting embryo implantation [17, 18]. It can also trigger thromboinflammation via complement activation and trophoblast apoptosis, impairing spiral artery remodeling and placental perfusion [19]. Additionally, ANA triggers endometrial inflammation by activating Toll-like receptor (TLR), and increasing TNF-α and IL-6 levels. These inflammatory factors impair decidualization, restrict trophoblast invasion through complement activation, and cause vascular endothelial dysfunction and microthrombosis [20–23].

Studies have confirmed that targeted immunomodulatory therapies can counteract the pathological reactions caused by these abnormal immune markers [23–25]. Heparin may exert a neutralizing effect by inhibiting β2-glycoprotein I binding and attenuating complement-mediated injury [23], whereas glucocorticoids may contribute to the restoration of homeostasis by reducing NK cell activity [24, 25]. Meanwhile, numerous studies have also reported that targeted therapies addressing abnormal antibody status, including treatment with prednisone and hydroxychloroquine, can improve both embryonic and pregnancy outcomes, even in patients who do not fulfill the diagnostic criteria for autoimmune diseases [26–28]. However, there is no consensus regarding the necessity of comprehensive immune marker screening before ART, particularly in the PGT population, where both embryos and transfer opportunities are limited and valuable. Furthermore, the benefit-risk ratio of empirical immunomodulatory therapy remains insufficiently supported by high-quality evidence in patients who exhibit normal immune markers but experience RPL or RIF. Specific guidelines are necessary for the analysis and treatment of patients who do not present a clear spectrum of autoimmune disorders.

Based on the above research background, the present study was designed as a retrospective cohort study, focusing on two core clinical questions: (1) whether early immunoscreening is necessary in the PGT population; (2) whether empirical immunomodulatory interventions (e.g., glucocorticoids, heparin) can improve pregnancy outcomes in PGT patients who present with normal immune parameters on routine immune screening. The results of this study will provide an important evidence-based foundation for the development of a precise immune assessment system and individualized treatment strategies.

Material and methods

Study design

This retrospective cohort study was conducted among patients undergoing PGT at the Reproductive Medicine and Genetics Center of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology between January 1, 2020 and December 31, 2024. This study was approved by the Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (Reference Number: TJ-IRB20211156). Written informed consent was obtained from all participants before their inclusion in the study, and all relevant data were ex-

tracted from the electronic medical record database.

Study population

Patients who underwent PGT and fulfilled the following inclusion and exclusion criteria were included in the study. Inclusion criteria: (1) age 20–45 years; (2) the second frozen embryo transfer (FET) performed within 6 months after the first failed PGT-FET; (3) completion of immune screening (ANA, antiphospholipid antibody profile, etc.) with no initiation of other treatment before the second transplantation; (4) transferred embryos refer to euploid embryos that have been identified through biopsy at the blastocyst stage. Exclusion criteria: (1) maternal anatomical abnormalities and severe medical conditions; (2) frozen embryos derived from different batches; (3) serious semen abnormalities in the male partner; (4) the utilization of donor sperm or oocytes.

Basis for grouping

Criteria for the stratification of immunization status: (1) positive group: individuals positive for any of the autoimmune disease screening markers; (2) negative group: all immune markers within the normal reference range. The negative group was further categorized into two subgroups: an empirical medication group, which received at least one form of immunomodulatory treatment (e.g., low-molecular-weight heparin, glucocorticoids), and a no-intervention group, which received only standard luteal phase support.

Definition of immune abnormalities

All immune markers were detected using routine clinical laboratory methods at our center. Immune abnormality was defined as the presence of any abnormal result among the following markers: (1) rheumatic panel: ANA titer $\geq 1:100$, according to institutional reference standards, with titers $\geq 1:320$ considered high-titer positivity; or any positive result among other autoantibodies in the rheumatic panel; (2) immunoglobulin and complement panel: elevated levels of IgG, IgM, or IgA, or decreased levels of complement C3 or C4, based on laboratory reference ranges; (3) lymphocyte subset analysis: increased proportions of natural killer (NK) cells or B lymphocytes beyond the upper limit of the laboratory reference range, as measured by flow cytometry; (4) cytokine detection: elevated serum levels of IL-6 or TNF-α. In addition to the markers listed above, other clinically relevant immune parameters assessed as part of routine practice in our center were also considered when available, and were interpreted according to institutional laboratory reference standards. This inclusive definition was adopted to reflect real-world clinical screening practices.

Immunotherapy regimen

For patients in the positive group, immunomodulatory therapy was administered based on the results of immunological marker screening, following a marker-oriented and semi-standardized treatment strategy. Specifically, patients with antiphospholipid antibody positivity were preferentially treated with low-molecular-weight heparin and/or aspirin, whereas those with antinuclear antibody positivity or evidence of inflammatory activation were more likely to receive glucocorticoids and/or hydroxychloroquine. In cases with multiple or high-titer abnormalities, combination therapy was considered. Treatment protocols were individualized according to each patient's immunological profile and clinical condition, and all medications were administered in accordance with standardized treatment strategies jointly developed by experienced immunologists and perinatal medicine specialists. In general, immunomodulatory therapy was initiated prior to embryo transfer and continued into early pregnancy. The duration of treatment was adjusted based on follow-up assessments of immune status and pregnancy progression. For example, low-molecular-weight heparin was typically continued until 10–12 weeks of gestation in most patients, while extended use until late pregnancy was considered in selected high-risk cases. Similarly, glucocorticoids were usually tapered after confirmation of clinical pregnancy. This approach allowed for individualized patient management while maintaining overall standardization of treatment strategies.

Endometrium preparation and embryo transfer

FETs were conducted in subsequent cycles using various endometrial preparation protocols. For women with regular menstrual cycles, follicular growth was monitored via transvaginal sonography (TVS) beginning on day 10 to 12 of the menstrual cycle. Oral dydrogesterone (30 mg/day) was generally initiated on the day of ovulation, followed by the transfer of a single euploid blastocyst 5 days after ovulation. For patients with irregular menstruation undergoing hormone replacement therapy (HRT), oral estradiol was administered on day 2 or 3 of the menstrual cycle and continued until the endometrial thickness reached ≥ 8 mm, after which endometrial transformation was initiated. For patients undergoing gonadotropin-releasing hormone agonist (GnRH-a) downregulation combined with HRT (GnRH-a + HRT), 3.75 mg of GnRH-a was administered subcutaneously on

days 1–2 of menstruation, and the HRT was started 28–30 days later to prepare the endometrium. Embryo transfer was carried out as described previously. Luteal support was continued until either 8–10 weeks of gestation or until a negative human chorionic gonadotropin (hCG) test result was obtained 2 weeks after embryo transfer, as previously described [29].

Outcome measurement

The primary outcome was defined as the live birth rate, which refers to the delivery of at least one infant after 28 weeks of gestation. Secondary outcomes included hCG positivity, clinical pregnancy rate, and miscarriage rate. hCG positivity was defined as a serum hCG level of ≥ 10 mIU/ml. Clinical pregnancy was confirmed by the presence of one or more gestational sacs in the uterus, as detected by TVS performed 4 weeks after embryo transfer. A miscarriage was defined as the loss of a pregnancy occurring before the 28th week of gestation.

Statistical analysis

The collected data were analyzed using SPSS version 27.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± SD or medians (first and third quartiles), depending on the normality of the distribution, while categorical variables were summarized as counts and percentages (%). The differences between the means of the two groups were compared using either the *t*-test or the Mann–Whitney test, depending on the appropriateness of the data distribution. Categorical variables were analyzed using the Pearson's χ^2 test or Fisher's exact test, as appropriate. Meanwhile, to explore the relationships among the variables, multivariate logistic regression models were employed. We calculated both crude regression estimates and estimates adjusted for relevant baseline covariates, along with corresponding 95% confidence intervals (CI). Two-tailed *p*-values < 0.05 were considered to indicate statistically significant [29].

Results

Baseline characteristics of the study population

This retrospective cohort study included 151 PGT patients who underwent autoimmune screening and experienced more than one embryo transfer. Among them, 42 patients were assigned to the positive group, while 109 patients were assigned to the negative group, which was further divided into two subgroups: 66 patients in the negative-no intervention group and 43 patients in the negative-intervention group (Figure 1). Baseline demographic and clinical characteristics, such as age, body mass index (BMI), and antral follicle count (AFC), did not significantly differ between the immune-positive

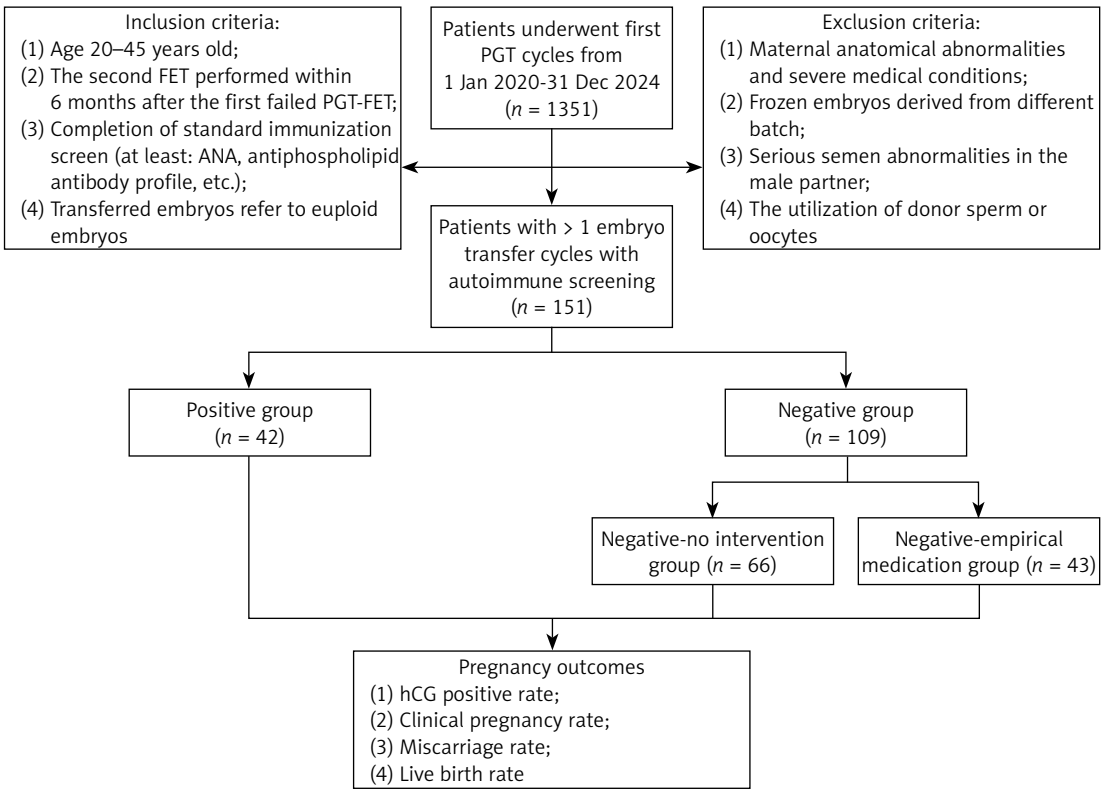

Figure 1. Study flowchart

PGT – preimplantation genetic testing, FET – frozen embryo transfer, aOR – adjusted odds ratio.

and immune-negative groups (Table I) or between the two immune-negative subgroups (Table II).

Clinical outcomes after embryo transfer

Patients with positive autoimmune screening who received targeted interventions before the second FET exhibited a significantly higher clinical pregnancy rate and a significantly lower miscarriage rate compared to their initial transfer, which was conducted without screening or intervention (64.29% vs. 33.33%, $p = 0.005$) (Table III). However, there was no statistically significant difference in the hCG positivity rate between the two transfer cycles (76.19% vs. 61.90%, $p = 0.157$) (Table III). In both immune-negative subgroups (empirical medication and no-intervention), the second FET cycle showed a significantly higher hCG positivity rate (74.24% vs. 39.39% and 74.42% vs. 37.21%, respectively, both $p < 0.05$) and clinical pregnancy rate (69.70% vs. 21.21% and 69.77% vs. 13.95%, respectively, both $p < 0.05$) compared to the first FET attempt. However, no statistically significant differences were observed in the miscarriage rate and live birth rate (13.33% vs. 19.57%, $p = 0.481$; 39.53% vs. 43.94%, $p = 0.649$) between the empirical medication and no-intervention subgroups for the second transfer (Table IV).

Univariate and multivariate logistic regression models were used to assess factors influencing pregnancy outcomes in the immune-negative group (Table V). After adjusting for potential confounders, multivariate regression analysis revealed that higher embryo quality (≥ 4BB) was an independent predictor of both hCG positivity (aOR = 3.03, 95% CI: 1.09–8.48, $p = 0.034$) and clinical pregnancy (aOR = 3.12, 95% CI: 1.18–8.29, $p = 0.022$). Increasing maternal age was negatively associated with these outcomes (aOR = 0.84, 95% CI: 0.73-0.97 for hCG positivity; aOR = 0.85, 95% CI: 0.75-0.97 for clinical pregnancy; aOR = 0.85, 95% CI: 0.74-0.97 for live birth, all $p < 0.05$). Empirical immunomodulation alone did not demonstrate significant independent effects on any outcomes after adjustment.

Table I. Baseline characteristics of the study groups

Variables	Positive group (*N* = 42)	Negative group (*N* = 109)	*P*-value
Age [years] M (Q_1, Q_3)	31.50 (29.00, 33.00)	31.00 (29.00, 34.00)	0.858
BMI [kg/m²] M (Q_1, Q_3)	21.48 (19.61, 25.23)	22.21 (20.20, 24.06)	0.609
AFC (number), M (Q_1, Q_3)	14.00 (11.00, 19.75)	13.00 (9.00, 19.00)	0.656
AMH [ng/ml] M (Q_1, Q_3)	4.04 (1.85, 6.40)	4.05 (2.84, 5.67)	0.582
FSH [mIU/ml], M (Q_1, Q_3)	6.59 (5.78, 8.38)	7.25 (6.17, 8.15)	0.187
PGT type, *n* (%)			0.213
PGT-A	24 (57.14)	63 (57.80)	
PGT-M	2 (4.76)	15 (13.76)	
PGT-SR	16 (38.10)	31 (28.44)	

*BMI – body mass index, AFC – antral follicle count, AMH – anti-Müllerian hormone, FSH – follicle-stimulating hormone, PGT-A – preimplantation genetic testing for aneuploidy, PGT-M – preimplantation genetic testing for monogenic disorders, PGT-SR – preimplantation genetic testing for structural rearrangements. *P < 0.05.*

Table II. Baseline characteristics of the immune-negative subgroups

Variables	Negative-no intervention group (*N* = 66)	Negative-empirical medication group (*N* = 43)	*P*-value
Age [years], M (Q_1, Q_3)	31.00 (29.00, 33.00)	32.00 (30.00, 34.00)	0.278
BMI [kg/m²], M (Q_1, Q_3)	22.52 (20.20, 24.23)	22.15 (20.33, 24.01)	0.951
AFC (number), M (Q_1, Q_3)	14.00 (10.00, 20.50)	13.00 (8.50, 18.50)	0.241
AMH [ng/ml], M (Q_1, Q_3)	4.21 (2.80, 5.81)	3.74 (2.88, 5.47)	0.745
FSH [mIU/ml], M (Q_1, Q_3)	7.37 (6.18, 8.18)	7.23 (6.39, 8.11)	0.998
PGT type, *n* (%)			0.848
PGT-A	37 (56.06)	26 (60.47)	
PGT-M	10 (15.15)	5 (11.63)	
PGT-SR	19 (28.79)	12 (27.91)	

*BMI – body mass index, AFC – antral follicle count, AMH – anti-Müllerian hormone, FSH – follicle-stimulating hormone, PGT-A – preimplantation genetic testing for aneuploidy, PGT-M – preimplantation genetic testing for monogenic disorders, PGT-SR – preimplantation genetic testing for structural rearrangements.*P < 0.05.*

Table III. Embryo transfer characteristics and clinical outcomes in the positive group

Variables	First embryo transfer (*N* = 42)	Second embryo transfer (*N* = 42)	*P*-value
Endometrial thickness, M (Q_1, Q_3)	8.80 (8.00, 9.40)	8.60 (7.82, 9.88)	0.531
Good-quality embryo, *n* (%)			0.221
< 4BB	9 (21.43)	14 (33.33)	
≥ 4BB	33 (78.57)	28 (66.67)	
Endometrial preparation, *n* (%)			0.009*
Downregulation + HRT cycles	3 (7.14)	11 (26.19)	
HRT cycles	35 (83.33)	25 (59.52)	
Natural cycles	4 (9.52)	2 (4.76)	
Ovulation induction cycles	0 (0.00)	4 (9.52)	
Pregnancy outcomes, *n* (%)			
hCG positive rate	26 (61.90)	32 (76.19)	0.157
Clinical pregnancy rate	14 (33.33)	27 (64.29)	0.005*
Miscarriage rate	14 (100.00)	1 (3.70)	
Live birth rate	–	23 (54.76)	

*HRT – hormone replacement therapy, hCG – human chorionic gonadotropin. *P < 0.05.*

Table IV. Embryo transfer information and clinical outcomes between immune-negative subgroups

Variables	No intervention group (*N* = 66)		*P*-value	Empirical medication group (*N* = 43)		*P*-value
	First embryo transfer (*n* = 66)	Second embryo transfer (*n* = 66)		First embryo transfer (*n* = 43)	Second embryo transfer (*n* = 43)	
Endometrial thickness, M (Q_1, Q_3)	8.90 (8.00, 9.45)	8.55 (8.00, 9.50)	0.641	8.70 (8.00, 9.75)	9.00 (7.90, 9.30)	0.789
Good-quality embryo, *n* (%)			0.829			0.149
< 4BB	14 (21.21)	13 (19.70)		9 (20.93)	15 (34.88)	
≥ 4BB	52 (78.79)	53 (80.30)		34 (79.07)	28 (65.12)	
Endometrial preparation, *n* (%)			< 0.001*			0.587
Downregulation + HRT cycles	7 (10.61)	17 (25.76)		10 (23.26)	14 (32.56)	
HRT cycles	58 (87.88)	36 (54.55)		28 (65.12)	22 (51.16)	
Natural cycles	1 (1.52)	7 (10.61)		3 (6.98)	3 (6.98)	
Ovulation induction cycles	0 (0.00)	6 (9.09)		2 (4.65)	4 (9.30)	
Pregnancy outcomes, *n* (%)						
hCG positive rate	26 (39.39)	49 (74.24)	< 0.001*	16 (37.21)	32 (74.42)	< 0.003*
Clinical pregnancy rate	14 (21.21)	46 (69.70)	< 0.001*	6 (13.95)	30 (69.77)	< 0.001*
Miscarriage rate	14 (100.00)	9 (19.57)		6 (100.00)	4 (13.33)	0.481
Live birth rate	-	29 (43.94)		-	17 (39.53)	0.649

*HRT – hormone replacement therapy, hCG – human chorionic gonadotropin. *P < 0.05.*

Table V. Univariate and multivariate regression analysis results of pregnancy outcomes in the immune-negative subgroups

Variables	hCG positivity				Clinical pregnancy				Miscarriage				Live birth			
	Univariate		Multivariate		Univariate		Multivariate		Univariate		Multivariate		Univariate		Multivariate	
	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	aOR (95% CI)	P	OR (95% CI)	P	aOR (95% CI)
Age [years]	0.030*	0.87 (0.77–0.99)	0.015*	0.84 (0.73–0.97)	0.038*	0.88 (0.78–0.99)	0.020*	0.85 (0.75–0.97)	0.706	0.97 (0.82–1.15)	0.681	0.96 (0.80–1.15)	0.038*	0.88 (0.79–0.99)	0.020*	0.85 (0.74–0.97)
BMI [kg/m^2]	0.610	0.96 (0.83–1.11)	0.901	0.99 (0.83–1.18)	0.752	0.98 (0.85–1.12)	0.999	1.00 (0.85–1.18)	0.893	1.01 (0.83–1.23)	0.820	0.97 (0.78–1.21)	0.255	0.93 (0.81–1.06)	0.381	0.94 (0.81–1.08)
Endometrial thickness	0.577	1.08 (0.82–1.42)	0.300	1.19 (0.86–1.65)	0.818	1.03 (0.80–1.33)	0.530	1.10 (0.82–1.49)	0.733	0.94 (0.65–1.35)	0.776	0.94 (0.63–1.42)	0.926	1.01 (0.80–1.28)	0.666	1.07 (0.80–1.43)
Good-quality embryo																
< 4BB		1.00 (Reference)		1.00 (Reference)		1.00 (Reference)		1.00 (Reference)		1.00 (Reference)		1.00 (Reference)		1.00 (Reference)		1.00 (Reference)
≥ 4BB	0.082	2.26 (0.90–5.69)	0.034*	3.03 (1.09–8.48)	0.050	2.44 (1.01–5.93)	0.022*	3.12 (1.18–8.29)	0.718	0.79 (0.22–2.80)	0.543	0.66 (0.17–2.52)	0.159	1.91 (0.78–4.71)	0.059	2.58 (0.96–6.89)
Endometrial preparation																
Downregulation + HRT cycles		1.00 (Reference)		1.00 (Reference)		1.00 (Reference)		1.00 (Reference)		1.00 (Reference)		1.00 (Reference)		1.00 (Reference)		1.00 (Reference)
HRT cycles	0.582	1.33 (0.48–3.72)	0.286	1.88 (0.59–6.04)	0.412	1.50 (0.57–3.92)	0.223	1.97 (0.66–5.86)	0.921	1.08 (0.25–4.64)	0.991	1.01 (0.21–4.74)	0.468	1.38 (0.57–3.34)	0.152	2.12 (0.76–5.90)
Natural cycles	0.161	0.35 (0.08–1.52)	0.231	0.35 (0.06–1.94)	0.316	0.48 (0.11–2.03)	0.340	0.45 (0.09–2.31)	0.132	4.00 (0.66–24.24)	0.135	4.52 (0.63–32.65)	0.093	0.15 (0.02–1.37)	0.154	0.19 (0.02–1.88)
Ovulation induction cycles	0.795	0.81 (0.17–3.92)	0.962	0.95 (0.14–6.34)	0.654	0.71 (0.16–3.11)	0.627	0.65 (0.12–3.65)	0.976	1.04 (0.10–11.25)	0.922	0.88 (0.07–11.65)	0.504	0.59 (0.13–2.74)	0.588	0.63 (0.12–3.39)
Group																
Empirical medication group		1.00 (Reference)		1.00 (Reference)		1.00 (Reference)		1.00 (Reference)		1.00 (Reference)		1.00 (Reference)		1.00 (Reference)		1.00 (Reference)
No intervention group	0.176	0.53 (0.21–1.33)	0.160	0.48 (0.17–1.33)	0.664	0.83 (0.36–1.93)	0.603	0.78 (0.31–1.97)	0.497	1.54 (0.44–5.35)	0.525	1.52 (0.42–5.52)	0.463	0.75 (0.34–1.62)	0.313	0.64 (0.27–1.52)

*CI – confidence interval, OR – odds ratio, aOR – adjusted odds ratio. *P < 0.05.*

Discussion

Successful embryo implantation and the maintenance of pregnancy depend on appropriate interactions between the embryo and the endometrium [30]. As previously discussed, immune-associated infertility remains a central topic in reproductive clinical research, with active debates regarding its underlying mechanisms and therapeutic strategies [31, 32]. Notably, current studies predominantly focus on exploring the immune status and the application of various immunotherapy regimens in the RPL population, yet their conclusions remain inconsistent [27, 33]. In contrast, for PGT patients, embryos are considered extremely valuable, making it crucial to enhance the success rate of embryo transfer [4]. Despite this imperative, the necessity of autoimmune screening and preemptive intervention remains underexplored in these patients. To address this gap, we conducted a cohort study to investigate pregnancy outcomes after two FET cycles involving single euploid blastocyst transfers in PGT patients who underwent autoimmune screening before second FET, as well as the potential impact of advanced immunologic intervention in mitigating adverse outcomes.

Our findings demonstrate a clear benefit of autoimmune screening for patients undergoing PGT who had experienced a first transfer failure. Among PGT patients with first transfer failure, 28% (42/151) showed abnormal immune markers, and targeted interventions based on screening results were associated with significantly improved clinical pregnancy rates in subsequent embryo transfer compared to their initial unscreened cycle (64.29% vs. 33.33%, $p = 0.005$). This finding aligns with results from previous studies [27, 28]. Surprisingly, our study found such a high prevalence of abnormal immune markers within the PGT cohort, which deserves clinical attention. Considering both the limited availability of embryos in PGT patients and the relatively high prevalence of immune abnormalities, comprehensive immune screening before the first embryo transfer may be warranted for this population. Early immune screening and targeted treatments may help reduce the risk of avoidable embryo loss and mitigate psychological trauma from repeated failures.

Notably, our findings revealed no significant benefit of empirical immunomodulatory therapy (e.g., corticosteroids or heparin) in immune-negative patients. No significant difference (39.53% vs. 43.94%, $p = 0.649$) was observed in live birth rate between immune-negative PGT patients who received empirical medication and those who received standard care alone. Importantly, multivariate analysis confirmed that empirical immunomodulation was not an independent predictor of improved clinical outcomes. Currently, there is ongoing debate regarding the effectiveness of empirical immune therapy. Some studies have confirmed that it can improve pregnancy outcomes [34, 35], while others have indicated that empirical therapy is ineffective [36, 37]. The divergent findings may result from differences in study populations and immunomodulatory intervention protocols. Previous studies did not exclude embryonic factors. By comparison, our PGT cohort excluded embryonic factors via genetic screening, shifting the focus to maternal status, thus making our conclusions more reliable. Therefore, based on the absence of therapeutic benefit and lack of independent predictive value in multivariate modeling in our study, the indiscriminate use of immune therapy in patients with immune-negative PGT failure does not appear to be justified.

In the multivariate analysis conducted within the immune-negative cohort, embryo quality (≥ 4BB) was identified as a strong positive predictor. Conversely, advancing maternal age was found to be a significant negative predictor of both hCG positivity and clinical pregnancy outcomes. This finding aligns with previous studies indicating that maternal age and embryo quality are key factors influencing in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) outcomes [38]. High-grade embryos (≥ 4BB) enhance implantation success by promoting optimal trophoblast invasion and placental development [39], whereas advanced maternal age is associated with mitochondrial dysfunction and compromised decidualization [40]. These findings call into question the increasingly common practice of administering broad-spectrum immune therapies to patients with PGT failures, highlighting the importance of prioritizing fundamental factors such as embryo selection and endometrial optimization.

Although PGT may reduce the risk of pregnancy failure due to embryonic factors, clinical practice shows that some PGT patients, particularly those with RPL, RIF, and advanced maternal age, still experience higher risk of conception failure or miscarriage. Consequently, an increasing number of studies are now focusing on the immune status and interventional therapies for this specific population. The necessity of autoimmune screening and preemptive intervention in PGT patients remains uncertain. Based on the findings of this study, we recommend that PGT patients undergo preemptive screening for autoimmune disease-related markers and receive targeted therapeutic interventions. In contrast, empirical immunomodulation may not be warranted for immune-negative PGT patients. Clinical efforts should prioritize optimizing embryo quality and addressing age-related endometrial dysfunction to prevent unnecessary medical interventions.

The strengths of this study include a well-defined and homogeneous PGT cohort that reflects real-world clinical conditions. However, this study also has several inherent limitations that should be acknowledged. First, the retrospective cohort design and before-after self-comparison limit causal inference regarding the therapeutic effect of targeted immunotherapy, and potential biases such as regression to the mean, unmeasured confounders, and variations in clinical management may affect the results. To address these issues, several measures were applied. Only PGT cycles with euploid embryo transfers were included, helping to reduce the confounding influence of embryonic factors. In addition, all patients were managed in a single center with relatively standardized endometrial preparation and transfer protocols, and adjustments in subsequent cycles were based on predefined clinical indications. Furthermore, multivariable regression models were used to adjust for key confounders. Nevertheless, residual confounding cannot be excluded, and the findings should be interpreted as associations rather than evidence of a causal relationship. Second, the immune-positive group included patients with heterogeneous immunological profiles, including autoantibody positivity, abnormal lymphocyte subsets, and elevated cytokines. Pooling these diverse immune abnormalities into a single group may obscure potential differences in treatment response. However, further stratification was not feasible due to limited sample size within specific subgroups, which could lead to reduced statistical power and unstable estimates. Third, this was a single-center study, which may limit the generalizability of the findings. Future well-designed prospective studies with larger sample sizes and multicenter collaboration are warranted to further clarify the role of targeted immunotherapy in PGT patients and to enable more refined stratified analyses.

In conclusion, this study presents evidence supporting the incorporation of autoimmune screening into the management protocols of PGT and demonstrates that empirical immunomodulatory therapy did not provide a significant benefit. Furthermore, clinical resources should be directed towards optimizing embryo quality and enhancing endometrial receptivity rather than implementing non-specific immune interventions in immune-negative patients. These findings provide insights that may inform the development of evidence-based and individualized treatment strategies for patients undergoing PGT.

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Ethical approval

This study was approved by the Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (Reference Number: TJ-IRB20211156).

Conflict of interest

The authors declare no conflict of interest.

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