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Trends in Immunotherapy

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Immunomodulatory Effects of Low-Dose Tacrolimus on Pro-Inflammatory and Anti-Inflammatory Cytokines in Women Undergoing Intracytoplasmic Sperm Injection

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Received: 20 July 2026; **Revised:** 10 August 2026; **Accepted:** 21 August 2026; **Published:** 23 September 2026

Abstract: Endometrial receptivity is regulated by sex hormones, growth factors, and cytokines. While the anti-inflammatory IL-10 promotes immunological tolerance, overexpression of the pro-inflammatory TNF-α inhibits implantation. Tacrolimus (FK506), a macrolide immunosuppressant, reduces pro-inflammatory cytokines by blocking calcineurin phosphatase. To assess the safety profile of periconceptional low-dose tacrolimus, including side effects and early pregnancy outcomes, and to ascertain how it influences clinical pregnancy rates in women undergoing fresh intracytoplasmic sperm injection (ICSI) cycles. From August 1, 2025, to July 1, 2026, Al-Nahrain University conducted this randomized interventional comparative study. The intervention (tacrolimus 1 mg twice daily from two days previous to embryo transfer until pregnancy testing, continued until 12 weeks if pregnant) and non-intervention groups were equally divided among 80 infertile women (20–40 years old) planned for new ICSI cycles. Serum TNF-α and IL-10 levels were assessed on the day of oocyte retrieval and embryo transfer. Rates of clinical pregnancy were calculated. $p < 0.05$ was considered significant. The intervention group had significant reductions in TNF-α (0.48 to 0.39, $p = 0.003$) and increases in IL-10 (0.35 to 0.49, $p < 0.001$). The Th1/Th2 ratio went back to normal from 2.20 to 0.81 ($p < 0.001$). Clinical pregnancy rates were greater in the intervention group (45% vs. 25%, $p = 0.061$). Tacrolimus produced no significant adverse effects and was well tolerated. By reducing TNF-α, boosting IL-10, and reestablishing Th1/Th2 balance, periconceptional low-dose tacrolimus successfully enhances endometrial receptivity with a positive inclination toward improved pregnancy rates. Low dosages of tacrolimus have a favorable safety profile.

Keywords: Th1/Th2 Balance; TNF-α; IL-10; ICSI; Clinical Pregnancy Rate; Endometrial Receptivity; Tacrolimus

1. Introduction

Infertility is the inability to conceive during a year or more of regular, unprotected sexual activity and may be a medical condition requiring treatment to become pregnant [1]. It affects a significant portion of couples globally and is recognized as a critical health issue [2,3]. By injecting a single sperm directly into the cytoplasm of a mature egg (metaphase II), ICSI gets over all natural barriers to fertilization, including the zona pellucida and oolemma [4]. This approach has revolutionized the treatment of male factor infertility since its successful introduction in 1992 [5,6].

Endometrial receptivity is attained when the endometrium reaches optimal cellular and molecular maturation during the secretory phase, which is clinically known as the window of implantation (WOI)—a short period of two to four days. Human embryo implantation is restricted to days 6–10 post-ovulation (days 19–23 of a 28-day cycle),

requiring significant vascular remodeling, the recruitment of uterine natural killer cells, and the differentiation of stromal fibroblasts into decidual cells [7,8]. Naive CD4+ T helper cells can evolve into Th1 (producing IFN-γ and TNF-α) or Th2 (producing interleukin-(IL)—4, IL-5, and IL-13) pathways, depending on the cytokine environment during antigen presentation [9]. A healthy pregnancy requires a shift toward Th2 immunity; regulatory T cells (Tregs) reduce Th1 responses through TGF-β and IL-10, while progesterone produces progesterone-induced blocking factor (PIBF). Reduced endometrial receptivity as a result of dysregulated adhesion molecules and increased uNK cell cytotoxicity, abnormal placentation with inflammatory and pro-thrombotic states, and direct cytotoxicity of the embryo and placenta are all consequences of Th1 dominance [10].

TNF-α, IL-10, and the Th1/Th2 ratio were selected because of their reciprocal functions in regulating reproduction immunity and their likely involvement in the immune-mediated phenomena of the perinatal implantation interval [11,12]. TNF-α is a critical pro-inflammatory determinant responsible for immune system triggering, inflammatory signaling processes, and the facilitation of cellular responses [13–15]. TNF-α is generated from activated macrophages and T lymphocytes [16,17]. It initiates and amplifies inflammatory responses by boosting cytokine production, leukocyte influx, and apoptosis in targeted cells [18–20]. Overactive TNF-α expression, although necessary for host defense, has been linked to chronic inflammatory/autoimmune disorders [21–24]. While managed inflammatory responses are a prerequisite for remodeling of tissues and the development of an optimal implantation setting, high levels of long-term TNF-related inflammation can give rise to undesirable immune conditions that may disrupt endometrial responsiveness and fetal-maternal relationships [25,26]. It may also interfere with implantation through stimulating tissue inflammation [27,28].

Conversely, IL-10 is an important anti-inflammatory and immunity-regulating cytokine involved in the inhibition of overactive inflammation and in the preservation of normal immunological tolerance [29, 30]. IL-10 is secreted by regulatory T cells (Treg), macrophages, and other immune systems [31–33]. It works to prevent immunological activation through suppressing inflammatory cytokine formation, limiting antigen expression, and increasing immune system tolerance [34–36]. In reproduction-related immunology, the regulatory effect of IL-10 is especially vital in preterm birth because maternal autoimmune reactions need to be properly managed to allow implantation and nurture the growing conceptus while not interfering with the essential mechanisms of host defense [37–39]. IL-10 promotes implantation and pregnancy via mitigating inflammation and preserving immunological homeostasis at the fetal-mother's junction [29,40].

The combined determination of TNF-α and IL-10 enables examination of pro-inflammatory and counter-regulating constituents of the overall immunological response versus an individual inflammatory biomarker [41,42]. Another aspect of systemic immune polarization is the Th1/Th2 balance. Th1-related cytokines are indicative of a heightened cellular-mediated pro-inflammatory phenotype, while Th2-dependent cytokines often correspond with humoral and immune-modulatory roles [43,44].

Variations in this balance are thus being examined in regard to maternal immunological tolerance, endometrial receptivity, implantation, and initial pregnancy maintenance. Vitally, proper implantation doesn't demand entire repression of inflammation but requires a highly controlled cycle of activated inflammatory processes accompanied by sufficient immune system resolution and tolerance [45,46]. Consequently, a concurrent measurement of TNF-α, IL-10, and the Th1/Th2 ratio offers supplementary data on the immune profile and can contribute to the characterization of the immunological parameters correlated with the peri-implantation duration and the possible immune-suppressive impacts of low-dose tacrolimus.

Tacrolimus, a potent macrolide calcineurin inhibitor, is one of the primary immunosuppressive drugs used to treat atopic dermatitis and prevent solid organ transplant rejection [47,48]. Because of its narrow therapeutic index, target trough levels typically range from 5 to 20 ng/mL depending on the indication, requiring stringent therapeutic drug monitoring [49,50]. It binds to FKBP12 and creates a complex that inhibits calcineurin phosphatase activity, blocking NFAT dephosphorylation and nuclear translocation, hence suppressing the transcription of IL-2, IL-3, IL-4, IFN-γ, and TNF-α [51,52]. Tacrolimus may reduce miscarriage and preterm labor in women with elevated Th1/Th2 ratios, prevent organ transplant rejection, and manage autoimmune diseases, particularly lupus nephritis, when conventional treatments are insufficient [53–55]. Among the negative consequences on mothers include hypertension, nephrotoxicity, and gestational diabetes. Fetal and neonatal concerns include preterm birth, low birth weight, transient neonatal hyperkalemia, renal dysfunction, and potential long-term neurodevelopmental problems. For obstetric uses, the therapeutic window is 3–7 ng/mL; the hazardous threshold is reached when trough levels

consistently above 15 ng/mL. Exposure to high dosages during the first trimester is linked to the highest risk of teratogenicity [56,57].

Goals of the Study

1. To look at how low-dose tacrolimus administered periconceptionally affects the implantation rate (IR) in women undergoing new ICSI cycles.
2. To assess how the clinical pregnancy rate (PR) in women undergoing new ICSI cycles is affected by periconceptional low-dose tacrolimus.
3. To evaluate the periconceptional safety profile of low-dose tacrolimus, considering possible side effects and early pregnancy outcomes.

2. Materials and Methods

2.1. Study Design

We conducted a prospective, randomized, open-label, parallel-group controlled trial at the High Institute for Infertility Diagnosis & Assisted Reproductive Technologies, Al-Nahrain University, Baghdad, from August 1, 2025, to July 1, 2026.

Eligible women commencing their first ICSI cycle were recruited and randomly allocated in a 1:1 ratio to either the tacrolimus group, who received low-dose tacrolimus along with the standard ICSI regimen, or the control group, who received the standard ICSI regimen alone.

Randomization assignment was electronically-generated from a randomization sequence created independently of the researchers performing enrollment of participants and clinical evaluation. Allocation concealment was preserved during enrollment procedures. Treatment responsibilities were announced following the applicant's being found eligible and registered in the study. This method of randomization and placement was used to avoid biased selection and to evenly distribute baseline features between research cohorts.

Tacrolimus's impact on implantation and pregnancy outcomes in women undergoing new ICSI cycles was the main goal. The local medical ethical committee approved the research procedure, and each participant provided signed informed permission.

Participants: Eighty infertile women between the ages of twenty and forty who were scheduled for fresh ICSI cycles participated in this study. Acute infection signs led to the elimination of five of the 85 women who were first evaluated. Participants were split into two groups of forty: the Intervention Group was given 1 mg of tacrolimus twice a day starting two days before the embryo transfer, while the Non-Intervention Group was not given any tacrolimus.

The research design was assessed and authorized by the Ethical Committee of the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies at Al-Nahrain University in Baghdad, Iraq. The ethical permission was given on August 1, 2025 (permission code: 0701-Df-2026R76). The study followed the Declaration of Helsinki's ethical principles as well as the relevant institutional rules. Before enrolling, all participants were given a full description of the project's aims, methods, anticipated advantages, and safety concerns. They were advised that their involvement was fully voluntary, that they could opt out from the investigation at any time without compromising their healthcare, and that their private and medical data would be kept strictly confidential. All subjects provided written informed consent before being included in the trial.

Women between the ages of 20 and 40, a diagnosis of primary or secondary infertility, causes such as male factor, tubal factor, unexplained infertility, or PCOS, an antagonist regimen for ovulation induction, and the availability of high-quality embryos for transfer on day three or day five are all requirements for inclusion.

Exclusion criteria include the presence of long-term systemic endocrine issues (such as diabetes mellitus or thyroid illness) or the use of immunosuppressants (including corticosteroids) within the preceding three months.

Intervention: Beginning two days before embryo transfer and continuing until pregnancy testing (about 16–18 days), the intervention group was given oral tacrolimus 1 mg twice daily. Tacrolimus was administered to patients who tested positive for pregnancy until 12 weeks of gestation.

Monitoring and Evaluations: Each participant had a biochemical evaluation on the day of oocyte retrieval, before to tacrolimus administration (**Figure 1**) and the day of fresh embryo transfer, after tacrolimus administration

(**Figure 2**). Serum TNF-α and IL-10 levels were measured using venous blood samples and the enzyme-linked immunosorbent assay (ELISA).

Figure 1. High Institute of Infertility Diagnosis and Assisted Reproductive Technologies: Oocytes Retrieved at Different Maturation Stages.

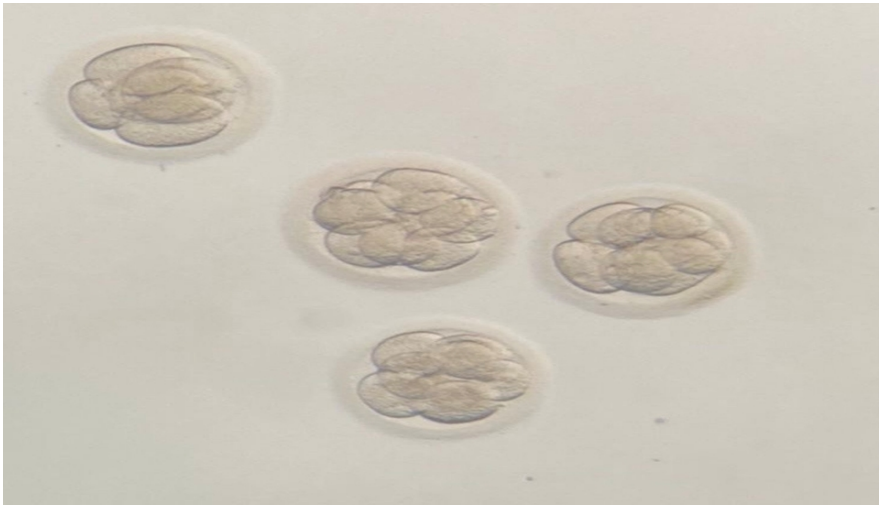

Figure 2. Developing Embryos at the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies.

Blood Collection & Processing: Venous blood samples were drawn using disposable syringes on the day of oocyte retrieval, put into sterile gel plastic tubes, and left to coagulate at 37 °C for 30 min [58,59]. Centrifugation at 1,000 rpm for 10 min was used to separate the serum, which was then kept at −20 °C for biomarker analysis.

Measures of Hormonal Outcomes: Sandwich enzyme immunoassay kits (ELISA) based on biotin double antibody sandwich technology were used to measure serum TNF-α level. TNF-α monoclonal antibody was used to pre-coat the wells. Biotin-labeled anti-TNF-α antibodies and streptavidin-HRP produced immunological complexes. The color shift at 450 nm was determined using a spectrophotometer. There was a positive correlation between color intensity and TNF-α concentration.

2.2. Measures of Inflammatory Outcomes

The serum levels of TNF-α and conventionally obtainable Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) kits conforming to the supplier's specifications. The ELISA depends on a particular antibody–antigen interaction paradigm, in which the targeted cytokine in the serum specimen attaches to designated antibodies that are stationary on the test plate and then identified with an enzyme-labeled detection antibody [60,61]. The enzyme-

mediated reaction produces a quantifiable colorimetric signal that is proportional to the level of the desired cytokine. Cytokine monoclonal antibody was used to pre-coat the wells. Biotin-labeled anti-IL-10 and anti-TNF-α antibodies and streptavidin-HRP formed immunological complexes [62,63]. The color shift at 450 nm was determined using a spectrophotometer. There was a positive correlation between color intensity and IL-10/TNF-α levels.

All samples, standards, and quality control materials have been examined following the prescribed assay protocols. Samples were measured in duplicate, and the mean of the duplicate values was employed for statistical analyses. Cytokine amounts were obtained from the appropriate standard curves derived from the calibrators that came with each ELISA.

The quality-control methods provided by the company were used to observe experiment accuracy. Throughout routine laboratory testing, individuals in charge of cytokine parameters were blind to the clinical group assignment.

2.3. Pregnancy Outcome

At least one intrauterine gestational sac with visible fetal heart activity on transvaginal ultrasound three to four weeks after ICSI was considered a clinical pregnancy. Fourteen days following ICSI, serum β-hCG levels were measured. The number of gestational sacs divided by the number of transplanted embryos yielded the implantation rate.

2.4. Compliance/Adherence

Patient self-reports and routine follow-up visits were used to track treatment adherence. Adherence statistics were recorded, and participants who showed poor adherence received counseling.

2.5. Reporting of Adverse Events

Safety surveillance was undertaken during the course of the treatment and the follow-up period. Treatment-related adverse events and clinically important manifestations have been evaluated during planned clinical appointments. Special consideration was paid to symptoms possibly linked to tacrolimus therapy, and regular laboratory and clinical examinations were investigated when appropriate. Negative outcomes have been documented and graded for degree of severity and correlation to assess intervention. All subjects of the research were followed until the end of the specified follow-up period.

2.6. Statistical Analysis

Microsoft Excel 2010 and SPSS version 23 were utilized. The Shapiro-Wilk test was used to assess normality. Normally distributed variables were displayed as mean ± SD, but non-normally distributed data were displayed as median (IQR). The independent samples t-test looked at means, whereas the Mann-Whitney U test compared medians. Chi-square or Fisher's exact test were used to compare proportions. Pregnancy outcome predictors were found using binary logistic regression. The threshold for statistical significance was fixed at $p < 0.05$.

3. Results

3.1. Initial Features

Mean age (31.90 ± 4.71 vs. 33.65 ± 4.57 years, $p = 0.095$), median infertility duration (9 vs. 8 years, $p = 0.169$), and mean BMI (28.23 ± 3.38 vs. 28.26 ± 2.40 kg/m^2, $p = 0.959$) did not differ significantly between the intervention and control groups at baseline. There were no significant changes ($p > 0.05$) in the distribution of infertility types and etiological reasons as shown in **Table 1**.

Table 1. General Characteristics of Infertile Women Enrolled in This Study.

Characteristic	Intervention Group n = 40	Non-Intervention Group n = 40	P
Age (years)			
Mean ± SD	31.90 ± 4.71	33.65 ± 4.57	0.095 N I
Infertility duration (years)			
Median (IQR)	9 (6.75)	8 (3.75)	0.169 N M

Table 1. *Cont.*

Characteristic	Intervention Group n = 40	Non-Intervention Group n = 40	P
BMI (kg/m^2)			
Mean ± SD	28.23 ± 3.38	28.26 ± 2.40	0.959 N I
Normal weight, n (%)	2 (5%)	8 (20%)	0.074 N C
Over weight, n (%)	26 (65%)	18 (45%)	
Obese, n (%)	12 (30%)	14 (35%)	
Infertility type			
Primary, n (%)	30 (75%)	36 (90%)	0.139 N F
Secondary, n (%)	10 (25%)	4 (10%)	
Cause of infertility			
Male factor	9 (22.5%)	15 (37.5%)	
Female factor	14 (35%)	15 (37.5%)	0.340 N C
Combined	7 (17.5%)	4 (10%)	
Unexplained	10 (25%)	6 (15%)	

Note: BMI: body mass index; N: not significant; S: significant; SD: standard deviation; I: independent samples student *t*-test; C: Chi-square test; F: Fischer exact test; M: Mann Whitney U test.

3.2. Levels of Basal Hormones

Hormonal comparability was confirmed by the lack of significant differences in baseline serum levels of FSH, LH, AMH, estrogen, TSH, and prolactin ($p > 0.05$ for all) as illustrated in **Table 2**.

Table 2. Comparison of Basal Serum Hormone Levels between Intervention and Control Groups.

Characteristic	Intervention Group n = 40	Non-Intervention Group n = 40	P
FSH (mIU/mL)			
Median (IQR)	9.6 (8.51)	11.55 (2.72)	0.225 N M
LH (mIU/mL)			
Median (IQR)	3.97 (1.87)	4.1 (1.25)	0.241 N M
AMH (ng/mL)			
Median (IQR)	1.15 (1.92)	1.2 (0.93)	0.862 N M
E_2 (pg/mL)			
Median (IQR)	39.81 (19.3)	41.05 (10.55)	0.271 S M
TSH (mIU/mL)			
Median (IQR)	2.01 (0.98)	1.88 (0.75)	0.345 N M
Prolactin			
Median (IQR)	16.9 (9.43)	18.3 (5.4)	0.124 N M

Note: IQR: inter-quartile range; M: Mann Whitney U test; N: not significant; S: significant; FSH: follicle stimulating hormone; LH: luteinizing hormone; E_2: estradiol; TSH: thyroid stimulating hormone.

3.3. Oocyte Features

Despite identical total and GV oocyte counts, the intervention group had considerably more mature MII oocytes [6 (6) vs. 4 (3), $p = 0.030$] and significantly less immature MI oocytes [0 (1) vs. 1.5 (3), $p = 0.003$] than controls as revealed in **Table 3**.

Table 3. Comparison of Oocyte Characteristics between Intervention and Control Groups.

Characteristic	Intervention Group n = 40	Non-Intervention Group n = 40	P
Oocyte count			
Median (IQR)	8 (6)	7 (7)	0.938 N M
MII oocyte			
Median (IQR)	6 (6)	4 (3)	0.030 S M
Range	1–11	2–13	
MI oocyte			
Median (IQR)	0 (1)	1.5 (3)	0.003 S M
GV oocyte			
Median (IQR)	0 (2)	0.5 (3)	0.225 N M

Note: IQR: inter-quartile range; M: Mann Whitney U test; N: not significant; S: significant; MII: meta-phase II; MI: meta-phase I; GV: germinal vesicle.

3.4. Immunological Indicators

TNF-α: Baseline readings were similar ($p = 0.847$). After the intervention, TNF-α significantly decreased in the intervention group from 0.48 to 0.39 ($p = 0.003$), whereas there was no significant change in the control group ($p = 0.173$). There was no significant difference in the percentage between the groups ($p = 0.143$) as illustrated in **Table 4**.

Table 4.

Table 4. Comparison of serum TNF-alpha before, after intervention, and between intervention and control groups.

Characteristic	Intervention Group n = 40	Non-Intervention Group n = 40	P
Pre-TNF alpha			
Median (IQR)	0.48 (0.38)	0.46 (0.44)	0.847 N M
Post-TNF-alpha			
Median (IQR)	0.39 (0.25)	0.42 (0.40)	0.316 N M
p-value	0.003 W S	0.173 W N	
Percent difference %			
Median (IQR)	−31.48 (37.37)	−10.19 (84.44)	0.143 N M

Note: TNF: tumor necrosis factor; IQR: inter-quartile range; M: Mann Whitney U test; N: not significant; W: Wilcoxon test.

IL-10: Baseline readings were similar ($p = 0.184$). In the intervention group, IL-10 significantly rose from 0.35 to 0.49 ($p < 0.001$), but controls showed no change ($p = 0.830$). There was a significantly significant percentage difference ($p < 0.001$) between the groups as shown in **Table 5**.

Table 5. Comparison of IL-10 before and after Intervention between Groups.

Characteristic	Intervention Group n = 40	Non-Intervention Group n = 40	P
Pre IL-10			
Median (IQR)	0.35 (0.20)	0.38 (0.46)	0.184 N M
Post IL-10			
Median (IQR)	0.49 (0.48)	0.35 (0.50)	0.289 N M
p-value	<0.001 W S	0.830 W N	
Percent difference %			
Median (IQR)	66.52 (109.61)	−8.42 (119.14)	<0.001 S M

Note: TNF: tumor necrosis factor; IQR: inter-quartile range; M: Mann Whitney U test; N: not significant; W: Wilcoxon test.

Th1/Th2 Ratio (TNF-α/IL-10): The intervention group had a significantly higher baseline ratio (2.20 vs. 0.99, $p = 0.031$). Following the intervention, the ratio fell from 2.20 to 0.81 ($p < 0.001$) in both the intervention group and the controls ($p < 0.001$). Ratios after the intervention were comparable ($p = 0.355$). There was a significant percent difference ($p = 0.006$)as revealed in **Table 6**.

Table 6. Comparison of Th1/TH2 Ratio before and after Intervention between Groups.

Characteristic	Intervention Group n = 40	Non-Intervention Group n = 40	P
Pre Th1/TH2 ratio			
Median (IQR)	2.20 (2.33)	0.99 (1.44)	0.031 S M
Post Th1/TH2 ratio			
Median (IQR)	0.81 (0.99)	0.80 (2.06)	0.355 N M
p-value	<0.001 S W	<0.001 S W	
Percent difference %			
Median (IQR)	−58.91 (78.47)	−15.02 (138.70)	0.006 S M

Note: TNF: tumor necrosis factor; IQR: inter-quartile range; M: Mann Whitney U test; N: not significant; W: Wilcoxon test.

3.5. Adverse Events

Throughout the trial duration, no noteworthy pharmacological adverse reactions were noted. No participant stopped therapy due to significant undesirable side effects, and the low-dose tacrolimus protocol was typically well

tolerated. During follow-up, clinical and laboratory security indices stayed within clinically reasonable limits.

3.6. Features of the Embryo

Day 5 blastocyst transfers were higher in controls (60% vs. 35%, $p = 0.025$), whereas Day 3 embryo transfers were considerably higher in the intervention group (65%) compared to controls (40%) as shown in **Table 7**.

Table 7. Comparison of Embryo Characteristics between Intervention and Control Groups.

Characteristic	Intervention Group n = 40	Non-Intervention Group n = 40	P
Embryo age			
Day 3	26 (65.0%)	16 (40.0%)	0.025 S
Day 5	14 (35.0%)	24 (60.0%)	C

Note: C: chi-square test; S: significant.

3.7. Pregnancy Results

Compared to the control group (25%, 10/40), the intervention group had a greater clinical pregnancy rate (45%, 18/40). The *p*-value of 0.061 was close to but fell short of traditional statistical significance as revealed in **Table 8**.

Table 8. Pregnancy Rate Comparison between Intervention and Control Groups.

Pregnancy	Intervention Group n = 40	Non-Intervention Group n = 40	P
Positive	18 (45.0%)	10 (25.0%)	0.061 N
Negative	22 (55.0%)	30 (75.0%)	C

Note: C: chi-square test; N: not significant.

3.8. Analysis of Logistic Regression

There were no notable variations among the investigated groups when speaking of age, BMI, infertility type/duration/causes, FSH, LH, AMH, E2, TSH, prolactin, oocyte count, MII oocyte count, and MI oocyte count, all of which did not substantially predict pregnancy outcome ($p > 0.05$). This reveals that the groups under study were reasonably similar in terms of the analyzed baseline demographic, clinical, hormonal, and oocyte-linked parameters, as illustrated in **Table 9**.

Table 9. Binary Logistic Regression Analysis Considering Pregnancy as the Dependent Variable.

Characteristic	*p*	Interpretation
Age	1.000	Not significant
BMI	0.992	Not significant
Infertility type	0.986	Not significant
Infertility duration	1.000	Not significant
Infertility causes	1.000	Not significant
FSH	0.988	Not significant
LH	0.981	Not significant
AMH	0.994	Not significant
E_2	0.990	Not significant
TSH	0.987	Not significant
Prolactin	0.992	Not significant
Oocyte count	0.999	Not significant
MII oocyte	0.999	Not significant
MI oocyte	0.996	Not significant

4. Discussion

Successful implantation requires a healthy embryo, a receptive endometrium, and "triple concordance"—coordinated molecular signaling [64–66]. This study shows that tacrolimus improves pregnancy outcomes by regulating the TNF-α/IL-10 ratio and altering the Th1/Th2 balance to an anti-inflammatory state. Controlled, transient inflammation is necessary for both pinopode formation and endometrial receptivity [67–69]. Critics argue that Th17/Treg balance is a more accurate measure of implantation success and that strict Th2 dominance may not always be beneficial as a slight Th1 response may increase trophoblast invasion [70,71]. Thus, it is uncertain whether

the Th1/Th2 axis is the most crucial immune target for therapy. The groups were similar in terms of age, BMI, duration, type, and causes of infertility ($p > 0.05$), lessening confounding and enable post-treatment changes to be linked to tacrolimus. Because age and BMI are powerful independent predictors of IVF/ICSI outcome, baseline comparability is crucial [72–74]. However, matching age and BMI alone may not guarantee full baseline equality [20,75,76].

Of note, the Th1/Th2 paradigm implemented in the current research constitutes just a single aspect of the complex immune system underlying reproductive adaptation. Growing proof points to the role of the Th17/Treg pathway in motherly immune modulation and the formation of a proper maternal–embryonic connection [77,78]. Th17 lymphocytes mainly synthesize IL-17A, IL-17F, IL-21, and IL-22 that augment inflammatory cascades, mobilize immune cell types, and support host defense [79–81]. However, increased Th17 cytokines may be congruent with a deleterious inflammation milieu during a prenatal period [82,83]. Instead, Treg cells secrete immunosuppressive cytokines, especially IL-10 and transforming growth factor-β (TGF-β) [84,85], which counteract disproportionate immune system activity and induce peripheral and mother-fetal immunologic tolerance [86,87].

The levels of FSH, LH, AMH, E2, TSH, and prolactin were similar in all groups. Because both groups started with similar hormonal profiles, differences in pregnancy outcomes can be attributed to the immunological treatment rather than hidden hormonal problems. This baseline matching is crucial because progesterone and estrogen regulate growth factors and endometrial preparation [88,89], while abnormal thyroid or prolactin impair endometrial function [90–92]. However, blood AMH levels may not necessarily correlate with uterine receptor activation, and single time-point investigations may miss dynamic hormonal changes [93].

Despite having a same total oocyte count, the intervention group had significantly more MII oocytes and fewer MI oocytes. Since inflammation lowers oocyte quality, the fact that tacrolimus was given after egg retrieval is more suggestive of pre-existing immunological problems than a direct pharmaceutical effect [94, 95]. Early embryos release signaling molecules that prime the endometrium, and poor embryo-endometrium communication lowers conception rates [25,96,97]. However, more mature eggs do not always guarantee high quality [98–100].

Regarding TNF-α, the baseline readings were similar. Following the intervention, TNF-α was considerably reduced in the treated group ($p = 0.003$) but remained stable in the controls. Lowering TNF-α is beneficial since high levels cause decidual cell death by inhibiting endometrial integrin β3 [101,102]. Immune-modulating drugs that reduce TNF-α boost live birth rates in RIF patients [103,104]. However, blood TNF-α may not correlate with endometrial levels [105,106], and TNF-α blocking may only benefit specific subgroups [28,107].

With respect to IL-10, the intervention group's levels were considerably higher ($p < 0.001$) than those of the controls. In addition to promoting Treg recruitment and spiral artery remodeling [16,108], IL-10 fosters maternal-fetal tolerance [12, 109]. But compared to TGF-β and IL-35, IL-10 might not have much of an impact [110], and excessive IL-10 might paradoxically reduce the uNK cells needed for vascular remodeling [111].

The Th1/Th2 ratio for the intervention group was lower at baseline (2.20 vs. 0.99, $p = 0.031$) but improved following treatment (0.81 vs. 0.80, $p = 0.355$). The percent change was substantially larger in the intervention group ($p = 0.006$). Correcting this ratio is clinically beneficial since increased Th1/Th2 predicts implantation failure [112,113]. Immune-modifying infusions that restore Th1/Th2 balance increase pregnancy outcomes in RIF patients [114,115]. However, sleep and stress cause daily fluctuations in the Th1/Th2 ratio [44,116], and large cohort studies have not identified a link between blood Th1/Th2 and live birth [117,118].

Concerning features of the embryo, Day 5 blastocysts were greater in the control group, whereas Day 3 transfers were higher in the intervention group (65% vs. 40%, $p = 0.025$). Even though Day 5 transfers are usually thought to be better, the intervention group had higher pregnancy rates, suggesting that tacrolimus overcome this disadvantage. Transferring Day 3 embryos may benefit women with immune issues by exposing them to the uterine environment earlier [119]. Regression analysis confirmed that the result of a pregnancy was unaffected by the stage of the embryo.

The clinical pregnancy rate was 45% in the intervention group compared to 25% in the control group ($p = 0.061$). Although the p-value was not statistically significant, the study might have been underpowered. If the Th1/Th2 ratio is adjusted, pregnancy rates ought to rise [43,44]. Critics argue that improvements in the endometrium may not be reflected in changes in blood cytokines and that this should be viewed negatively [120,121]. However, the pregnancy rate trend and significant cytokine changes constitute a regular pattern.

The current results ought to be regarded in the context of the larger, growing area of reproductive immunotherapy. Numerous immune-modulatory techniques were studied in women receiving assisted reproductive care, in-

volving corticosteroids, intravenous immunoglobulin, lipid-derived treatments, granulocyte colony-stimulating factor, and calcineurin inhibitors like tacrolimus [122–125]. Meanwhile, the scientific backing for these therapies is still varied, with significant discrepancies between trials in patient choices, immunological abnormality characterization, therapy duration, dose, and reproductive goals [126,127]. Tacrolimus has sparked considerable attention due to its potential to regulate T-cell stimulation and inflammatory signaling pathways [128–130]. Nonetheless, alterations in systemic inflammatory indicators do not always result in superior implantation, clinical pregnancy, or live birth rates [125,131,132]. As a result, the possible significance of tacrolimus deserves to be investigated in well-planned randomized studies that include standardized immunological phenotypes and clinically relevant reproductive findings.

Pregnancy outcome was not predicted by any baseline characteristics ($p > 0.05$), suggesting that tacrolimus is the cause of differences and that randomization was successful. The lack of a predictive effect for age and BMI, two important independent indicators of IVF success [133,134], indicates that the groups were well-matched. Critics note that unmeasured variables including genetic variations, endometrial microbiome, or prior cesarean history could lead to bias [135]. Equal serum AMH may not guarantee endometrial equivalence [136], and endometrial progesterone resistance may develop even in the presence of identical baseline hormones [137]. Despite these criticisms, logistic regression provides crucial evidence that randomization was successful.

Future research should include large-scale randomized controlled trials to confirm the potential pregnancy rate benefit of tacrolimus. Another significant drawback of the current work is that TNF-α and IL-10 amounts, along with the Th1/Th2 ratio, were measured in peripheral blood, not in the endometrial tissue itself. Circulatory cytokines could offer useful data about systemic immune function; however, their levels might not completely capture the multifaceted and location-governed immune milieu at the embryonic-maternal junction. There are several cellular and molecular elements of regional endometrial immunological control that could potentially be specifically monitored from the body's circulation. Thus, these results ought to be considered as confirmation of the systemic immune-modulatory impacts of low-dose tacrolimus, instead of as tangible proof of alterations in endometrial cytokine formation. Further investigations are warranted to explore localized immunity by evaluating endometrial biopsy material or different clinically relevant uterine specimens, such as tissue-specific cytokine synthesis, immunological cell identification with immunohistochemical techniques, molecular tests, or tissue-dependent protein measurement. Clinical studies should also investigate both the Th1/Th2 and Th17/Treg axes to determine their relative clinical significance in recurrent implantation failure, and establish the optimal tacrolimus dosage that maximizes therapeutic efficacy while minimizing adverse effects.

While a reduced-dose tacrolimus corresponded with substantial differences in the immune-related indicators measured, such results failed to correlate with a statistically substantial rise in the clinical pregnancy rates. Hence, the reported immune-mediated implications ought not to be considered as solid proof of better fertility results. However, the immune responses observed are possibly favorable and recommend additional research in randomized trials with clinical pregnancy and live births as pre-specified main outcomes, conducted appropriately. More trials will be necessary to establish whether a suppression of immune biomarkers yields clinically meaningful systemic benefits in implantation and pregnancy effects.

The safety data offer initial confidence about the tolerance of the low-dose tacrolimus program employed in this trial. Nonetheless, the small sample size and period of follow-up hinder the capability to recognize rare or late-onset adverse effects. Additional trials with systematic liver, kidney, metabolic, pulmonary, blood-pressure, and other clinically pertinent safety assessments are necessary before reaching deeper conclusions about the safety of tacrolimus during assisted reproduction.

5. Conclusion

The findings of this study suggest that tacrolimus enhances endometrial receptivity in women with recurrent implantation failure by increasing IL-10, reducing TNF-α, and restoring the Th1/Th2 immune balance toward a more pregnancy-supportive profile, particularly in those with a pro-inflammatory immune phenotype. Although the observed increase in pregnancy rate did not reach statistical significance, the immunological improvements indicate that tacrolimus may represent a promising targeted therapeutic option for carefully selected patients with documented Th1/Th2 imbalance rather than for routine use in all IVF candidates. Baseline assessment of immune biomarkers, such as the Th1/Th2 or TNF-α/IL-10 ratio, may help identify women most likely to benefit from this

treatment.

Author Contributions

Conceptualization, methodology, investigation, funding acquisition, formal analysis, data curation, and writing—original draft, R.Y.J.; conceptualization, supervision, methodology, validation, resources, writing—review and editing, and final approval of the manuscript, M.T.A.-O.; supervision, validation, project administration, writing—review and editing, and final approval of the manuscript, L.A.A.-A. All authors have read and agreed to the published version of the manuscript.

Funding

The current study was not awarded any kind of funding.

Institutional Review Board Statement

The Ethical Committee of the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies at Al-Nahrain University examined and approved the study protocol. The ethical approval was issued on August 1, 2025 (approval Number: 0701-Df-2026R76). The research project followed the Declaration of Helsinki's ethical principles and the necessary institutional regulations.

Informed Consent Statement

Written informed consent was received from all women before enrollment.

Data Availability Statement

The data supporting the results of the present research are accessible from the corresponding author upon appropriate request.

Acknowledgments

The authors extend their sincere thanks to Al-Nahrain University for their invaluable support.

Conflicts of Interest

The authors declare no conflict of interest.

AI Use Statement

The authors declare that no artificial intelligence (AI) tools were used in the preparation of this manuscript.

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