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T cell subsets and their signature cytokines in infertile women prepared to undergo in vitro fertilization (IVF)

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Abstract

Background and objective: This study aimed to explore the immunological profile, particularly the balance between T helpers 1 (Th1), Th2, Th17, and regulatory T (Treg) cytokine responses, in women undergoing in vitro fertilization (IVF), and how these responses correlate with reproductive outcomes. It investigated the systemic levels of specific cytokines to evaluate their roles in implantation success and potential immunological barriers to fertility.

Methods: A case-control study was conducted at private IVF centers in Erbil city, Iraq. Peripheral blood samples were collected and analyzed using the Human Th1/Th2/Th17/Treg Multiplex Enzyme Linked Immuno-Sorbent Assay (ELISA) Kit to quantify interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), interleukin-2 (IL-2), IL-4, IL-13, IL-17, IL-22, and IL-10 levels.

Results: Significant elevations were found in Th2 cytokines (IL-4 and IL-13) and Treg cytokine (IL-10) in IVF patients compared to healthy controls ($P < 0.01$). Th1 (IFN-γ, TNF-α, IL-2) and Th17 (IL-17, IL-22) cytokines were modestly reduced, but these differences were not statistically significant ($P > 0.05$). Notably, anti-Müllerian hormone (AMH) levels were significantly higher in smokers, and luteinizing hormone (LH) levels were significantly elevated in women with prior IVF attempts.

Conclusion: The cytokine profile suggests an immune shift toward a Th2/Treg-dominant environment in IVF patients, supporting tolerance and embryo implantation. However, excessive skewing could impair local immune readiness. The findings highlight the immunological complexity of IVF outcomes and suggest potential benefits of targeted cytokine modulation to improve reproductive success.

Keywords: IVF, Cytokines, T helper cells, Regulatory T cells, ELISA.

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Introduction

Infertility is a major public health issue that affects 10 to 15% of couples of reproductive ages around the world (1). One of the most common treatments is in vitro fertilization (IVF), which isa complicated process where fertilization happens outside the body, followed by placing the embryo in the uterus. Despite advances in technology, the success rates of IVF remain low. About 30 to 40% of IVF cycles lead to live births, depending on factors like maternal age (2). While embryo quality, endometrial receptivity, and hormonal support are important for IVF success, there is growing interest in the role of the maternal immune system, especially the balance between T helper (Th) cell types and regulatory T (Treg) cells, in helping with implantation and early pregnancy development (3). Th1 cells release interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), and interleukin-2 (IL-2). Th2 cells provide IL-4 and IL-13. Th17 cells generate IL-17 and IL-22, while Tregs produce the anti-inflammatory cytokine IL-10 (4). The balance of these cell types is crucial for the success of pregnancy, especially in IVF situations. Th1 cells are key for cellular immunity and are important for fighting off intracellular pathogens. Their cytokines, especially IFN-γ, TNF-α, and IL-2,activate macrophages and heighten inflammatory responses. However, during pregnancy, an overactive Th1 response can be harmful. High levels of IFN-γ and TNF-α can lead to trophoblast cell death, poor remodeling of spiral arteries, and, ultimately, failure to implant or repeated pregnancy loss (RPL) (5). IL-2 is essential for T cell growth but can also worsen immune rejection if not kept in check (6). Research shows that women with RPL or multiple IVF failures have increased Th1 activity, particularly higher IFN-γ levels and an imbalanced Th1/Th2 cytokine ratio as affecting placental development (7). On the other hand, Th2 cells support humoral immunity and immune suppression, both of which are beneficial during pregnancy. IL-4 and IL-13, the key cytokines produced by Th2 cells, boost B cell activity, promote antibody production, and inhibit Th1 cell development. Their presence encourages maternal immune tolerance to fetal antigens and aids in trophoblast invasion (8). In successful IVF cycles and natural pregnancies, researchers often find a Th2-dominant profile, especially in the first trimester. Studies show increased levels of IL-4 and IL-13 in the endometrial fluid and serum of women who have positive IVF results. Therefore, boosting Th2 responses has been suggested as a way to improve implantation rates and lower early pregnancy loss in assisted reproductive processes (9). Th17 cells, a pro-inflammatory type of cluster of differentiation 4 (CD4+ T cell), produce IL-17 and IL-22, which are involved in

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autoimmune inflammation, attracting neutrophils, and maintaining epithelial barrier function. While they play an important role in mucosal immunity, heightened Th17 activity is closely linked to negative pregnancy outcomes (10). In IVF patients with recurrent implantation failure (RIF) or endometriosis, researchers have found increased levels of IL-17 and IL-22 in both blood and the endometrial tissue (11). IL-17 can interfere with the maternal-fetal relationship by promoting harmful inflammation, hindering trophoblast invasion, and immune rejection. While IL-22 has protective roles, it can also worsen inflammation under certain conditions (12). Tregs help balance the inflammatory responses of Th1 and Th17 cells. They are defined by the expression of CD4, CD25, and the FoxP3 transcription factor. Tregs provide immunosuppressive effects by producing IL-10 and TGF-β, both of which are essential for maintaining immune tolerance during pregnancy (13). IL-10 particularly plays a critical role in reducing the activity of effector T cells, lowering cytokine storms, and protecting trophoblasts from immune attack. Numerous studies show that higher levels of Tregs and increased IL-10 are related to successful implantation and continuing pregnancy, whether through natural conception or IVF (14). In contrast, a lack of Tregs or their impaired function has been noted in women with RPL, autoimmune infertility, and unsuccessful IVF cycles (15). Cytokine profiles in these instances usually show high levels of IL-4, IL-13, and IL-10, along with low levels of IFN-γ, TNF-α, IL-2, IL-17, and IL-22 (16). The aim of the present study is to find out the relation between successful rates of IVF with T cell representing cytokines levels accordingly.

Materials and Methods

Strategy of sample Collection and storage

The Pharmacy College of Hawler Medical University, Erbil, Iraq, Human Ethics Committee approved this study (No. HMU-EC-Ph-22/9/2024-29). Each patient gave written agreement to share data in this study. This study was conducted at Erbil City/Iraq IVF clinics.Thiscase control study examined blood samples from miscarriage and RIF patients. Every instance agreed to use their samples as inclusion criteria. Direct interviews provided socio-demographic and clinical data. All samples were taken sterilely to avoid contamination. All contributing centre blood samples were processed similarly. IVF patients provided samples before the treatment. Eight ml of peripheral blood was drawn using normal phlebotomy techniques. Blood was collected in a gel tube without anticoagulant and coagulated at room temperature for 5–10 minutes. After centrifugation at 6000 rpm for 5 minutes, sera were divided, aliquoted,

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frozen, and kept at −80°C (ultra-low freezer) in IVF centre. Samples could only be freeze-thawed twice.

Blood tests

A total of one hundred and three (103) cases signed the consent form and agreed to participate in such study for research purposes only. Whole blood patient samples have been collected at Dr. Atyaf's Centre for IVF in Soran Hospital in Erbil City. The samples were collected from those patients who have the incapability of having normalpregnanciesandanalysedfor thyroid function tests, hormonal assays, and anti-mullerian hormone (AMH).

Immunoassay Multiplex

IFN-γ, IL-2, IL-4, IL-10, IL-13, IL-17, IL-22, and TNF-αcan be semi-quantified in serum samples used Human Th1/Th2/Th17/Treg MultiplexELISA Kit. (Product of Arigo Biolaboratories Corporation, (Zhubei, Taiwan) catalogue number ARG83516). This semi-quantitative sandwich enzyme immunoassay(BioTekELx800) microplatereaderwas used (Winooski, Vermont, USA). IFN-γ, IL2, IL4, IL10, IL13, IL17A, IL22, and TNF-α antibodies were pre-coated on microtiter plate wells. Target cytokines bind to immobilised particular antibodies in wells after standards, mixes, and samples are pipetted in.

Statistical analysis

Data are presented as mean ± standard deviation (SD). Statistical significance was set at $P < 0.05$. Data were analyzed using GraphPad Prism 10 version X. Spearman's Rho and Pearson Correlation was used to measure correlations between variables like age, BMI, economic status, hormonal parameters, etc. Mann-Whitney U Test was applied for non-normally distributed data (e.g., hormonal variables like E2 and AMH between groups). Independent t-tests was used for comparing the means of normally distributed data (e.g., LH levels between smokers and non-smokers).

Results

As mentioned previously 103 cases have been participated in this investigation and agreed to be use their data for research purposes only. The average age of participants was 33.26 years (SD: 5.97), with an average height of 164.86 cm (SD: 7.40), weight of 67.92 kg (SD: 7.68), and BMI of 24.96 (SD: 1.95). A majority reported a high economic status (98.1%), and most were non-employed (54.4%). The majoritywere educated (88.3%), while 57.3% were smokers and 22.3% had chronic diseases.In term of reproductive health, all participants had cycles, with 56.3% having regular ones. Miscarriages were common, reported by 84.5% of participants, and 59.2% had previous IVF treatments. More participants had pregnancies without IVF (59.2%)

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compared to those with IVF (40.8%). IVF attempts were made by 23.3% of participants, while 76.7% had not undergone IVF. Drug use was reported by 36.9%, and only 3.9% had immune diseases. The hormonal profile showed a mean estradiol (E2) level of 78.62 pg/mL (SD: 155.99), progesterone at 0.62 ng/mL (SD: 2.93), luteinizing hormone (LH) at 5.23 IU/L (SD: 2.46), prolactin at 15.92 ng/mL (SD: 9.90), follicle-stimulating hormone (FSH) at 7.46 IU/L (SD: 3.38), free T3 (FT3) at 4.64 pmol/L (SD: 0.73), free T4 (FT4)at 1.11 pmol/L (SD: 0.23), thyroid-stimulating hormone (TSH) at 2.52 mIU/L (SD: 1.57), and anti-Müllerian hormone (AMH) at 2.53 ng/mL (SD: 2.01). Beta-HCG levels were undetectable (0.1 mIU/mL). These data provide a comprehensive profile of the participants' demographic, health, and reproductive characteristics.A detailed summary of the demographic, health, and reproductive characteristics of the study participants, along with their hormonal levels has been presented in Table 1.

Regarding parity, no significant difference was found between smokers (mean: 12.49, SD: 0.82) and non-smokers (mean: 12.80, SD: 0.93), with a P-value of 0.079. For estradiol (E2), smokers had a higher mean (97.38, SD: 202.65) compared to non-smokers (53.47, SD: 34.73), but this difference was not significant (P = 0.697). Progesterone levels were also higher in non-smokers (mean: 1.01, SD: 4.48) than in smokers (mean: 0.33, SD: 0.26), with a P-value of 0.103, indicating no significant difference.

For luteinizing hormone (LH), prolactin, follicle-stimulating hormone (FSH), free T3 (FT3), free T4 (FT4), and thyroid-stimulating hormone (TSH), no significant differences were observed between smokers and non-smokers, with P-values ranging from 0.466 to 0.878. However, there was a significant difference in anti-Müllerian hormone (AMH) levels, with smokers having higher mean values (2.89, SD: 2.18) compared to non-smokers (2.03, SD: 1.65), and a P-value of 0.027, indicating statistical significance. The statistical tests used were Mann-Whitney U tests (⸸) for most variables and t-tests for others. The comparison of hormonal and parity variables between smokers and non-smokers, showing the mean values, standard deviations, and P-values for each variable has been illustrated in Table 2.

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Table 1. Demographic, Health, and Reproductive Characteristics of Study Participants

		Mean	Standard Deviation	Frequency	%
Age		33.260	5.972		
Height		164.860	7.402		
Weight		67.920	7.683		
BMI		24.958	1.951		
Self-perceived economic status	medium			2	1.9
	high			101	98.1
Occupation	Employee			47	45.6
	Non-employee			56	54.4
Education level	educated			91	88.3
	Non-educated			12	11.7
Smoker	Yes			59	57.3
	No			44	42.7
Chronic diseases	Yes			23	22.3
	No			80	77.7
Cycles	Yes			103	100
Regular cycles	Yes			58	56.3
	No			45	43.7
Miscarriage	Yes			87	84.5
	No			16	15.5
IVF before	Yes			61	59.2
	No			42	40.8
IVF attempt	Yes			24	23.3
	No			79	76.7
Previous pregnancy	with IVF			42	40.8
	without IVF			61	59.2
Parity		12.621	0.876		
Drugs	Yes			38	36.9
	No			65	63.1
Immune diseases	Yes			4	3.9
	No			99	96.1
E2		78.621	155.999		
progesterone		0.618	2.933		
LH		5.228	2.464		
prolactin		15.916	9.904		
FSH		7.462	3.376		
FT3		4.639	0.730		
FT4		1.108	0.233		
TSH		2.523	1.568		
Beta HCG		0.100	0.000		
AMH		2.525	2.007		

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Table 2. Comparison of Hormonal and Parity Variables Between Smokers and Non-Smokers

Categories	Smoker	N	Mean	Std. Deviation	P-value
parity	Yes	59	12.492	0.817	0.079⸸
	No	44	12.795	0.930	(NS)
E2	Yes	59	97.381	202.645	0.697⸸
	No	44	53.466	34.728	(NS)
Progesterone	Yes	59	0.328	0.259	0.103⸸
	No	44	1.008	4.476	(NS)
LH: luteinizing hormone	Yes	59	5.382	2.435	0.466t
	No	44	5.022	2.516	(NS)
Prolactin	Yes	59	15.525	9.596	0.878⸸
	No	44	16.441	10.390	(NS)
FSH: follicle-stimulating hormone	Yes	59	7.248	3.297	0.208⸸
	No	44	7.749	3.496	(NS)
FT3	Yes	59	4.610	0.743	0.649 t
	No	44	4.677	0.719	(NS)
FT4	Yes	59	1.096	0.238	0.532 t
	No	44	1.125	0.227	(NS)
TSH: thyroid-stimulating hormone	Yes	59	2.627	1.661	0.503⸸
	No	44	2.385	1.441	(NS)
AMH: anti-Müllerian hormone	Yes	59	2.893	2.176	0.027⸸
	No	44	2.032	1.654	**Significant**

⸸: Mann-Whitney U, t: t-test two sample independent

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In terms of parity, there was no significant difference between the groups, with participants who had undergone IVF having a mean of 12.69 (SD: 0.89) and those who had not a mean of 12.52 (SD: 0.86), resulting ina P-value of 0.281. For estradiol (E2), the mean was higher in participants who had IVF before (87.94, SD: 192.78) compared to those who had not (65.08, SD: 76.43), but the difference was not significant (P = 0.781). Progesterone levels were lower in those who had IVF (mean: 0.35, SD: 0.24) compared to those who hadn't (mean: 1.00, SD: 4.59), with a P-value of 0.204, showing no significant difference.

For luteinizing hormone (LH), there was a significant difference, with participants who had IVF previously showing higher levels (mean: 5.64, SD: 2.62) compared to those who had not (mean: 4.62, SD: 2.10), with a P-value of 0.039. There were no significant differences for prolactin, follicle-stimulating hormone (FSH), free T3 (FT3), free T4 (FT4), thyroid-stimulating hormone (TSH), and anti-Müllerian hormone (AMH), with P-values ranging from 0.076 to 0.799. The statistical tests used were Mann-Whitney U tests (⏚)for most variables and t-tests for others. Tocompares of hormonal and parity variables between participants who had undergone previous IVF treatment and those who had not, presenting the mean values, standard deviations, and P-values for each variable has been illustrated in Table 3.

The analysis includes reproductive hormones (E2, progesterone, LH, prolactin, FSH), thyroid function tests (FT3, FT4, TSH), and the ovarian reserve marker AMH. Most parameters, such as parity, E2, progesterone, LH, FSH, thyroid hormones, and AMH, showed no significant differences between the two groups based on statistical tests like the Mann-Whitney U and independent t-tests. However, prolactin levels were significantly higher in women with prior pregnancies (P = 0.014), indicating a notable physiological difference potentially linked to lactation or postpartum hormonal changes. The findings demonstrate that previous pregnancy has little effect on most hormonal and thyroid markers, with prolactin being the only exception. The significantly elevated prolactin levels in women with prior pregnancies may reflect changes related to pregnancy and lactation. These results emphasize the overall stability of hormonal profiles across the groups, apart from prolactin, which warrants further investigation. The compactions of the differences in hormonal and parity variables between women with and without a previous pregnancy has been demonstrated in Table 4.

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Table 3. Comparison of Hormonal and Parity Variables Between IVF before

	IVF before	N	Mean	Std. Deviation	P-Value
Parity	Yes	61	12.690	0.886	0.281[ɻ] (NS)
	No	42	12.520	0.862	
E2	Yes	61	87.944	192.775	0.781[ɻ] (NS)
	No	42	65.081	76.427	
Progesterone	Yes	61	0.353	0.241	0.204[ɻ] (NS)
	No	42	1.004	4.588	
LH	Yes	61	5.643	2.624	0.039[t] Significant
	No	42	4.624	2.098	
Prolactin	Yes	61	16.102	10.139	0.799[ɻ] (NS)
	No	42	15.647	9.666	
FSH	Yes	61	7.823	4.086	0.267[ɻ] (NS)
	No	42	6.938	1.856	
FT3	Yes	61	4.677	0.795	0.528[t] (NS)
	No	42	4.584	0.629	
FT4	Yes	61	1.124	0.223	0.399[t] (NS)
	No	42	1.085	0.248	
TSH	Yes	61	2.674	1.555	0.108[ɻ] (NS)
	No	42	2.304	1.579	
AMH	Yes	61	2.399	2.206	0.076[ɻ] (NS)
	No	42	2.709	1.685	

[ɻ]: Mann-Whitney U, [t]: t-test two sample independent

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Table 4. Comparison of Hormonal and Parity Variables Between previous pregnancy

	Previous pregnancy	N	Mean	Std. Deviation	P-Value
Parity	Yes	42	12.690	0.897	0.471[ɻ]
	No	61	12.570	0.865	(NS)
E2	Yes	42	74.643	184.931	0.234[ɻ]
	No	61	81.360	134.097	(NS)
Progesterone	Yes	42	0.383	0.265	0.109[ɻ]
	No	61	0.780	3.809	(NS)
LH	Yes	42	5.940	2.781	0.109[t]
	No	61	4.737	2.107	(NS)
Prolactin	Yes	42	16.964	11.313	0.014[ɻ]
	No	61	15.195	8.830	Significant
FSH	Yes	42	8.077	4.579	0.452[ɻ]
	No	61	7.039	2.145	(NS)
FT3	Yes	42	4.672	0.836	0.411[t]
	No	61	4.616	0.653	(NS)
FT4	Yes	42	1.128	0.249	0.703[t]
	No	61	1.095	0.223	(NS)
TSH	Yes	42	2.596	1.632	0.487[ɻ]
	No	61	2.473	1.534	(NS)
AMH	Yes	42	2.631	2.317	0.747[ɻ]
	No	61	2.453	1.779	(NS)

[ɻ]: Mann-Whitney U, [t]: t-test two sample independent

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T cells subsets pay a crucial role in succussing IVF technique, therefore the variations between T cell subsets according to their specific cytokines between healthy women and those who undergoing IVF procedure have observed a significant difference in Th2 and Treg cells. Although cytokine levels appeared slightly lower in IVF patients compared to healthy controls, the differences were not statistically significant (P>0.05 for all comparisons). IFN-γ levels remained relatively consistent across groups, with IVF patients showing only a minor reduction (~55 pg/mL) compared to healthy individuals (~57 pg/mL). TNF-α concentrations decreased modestly from ~6.0 pg/mL in healthy subjects to ~4.5 pg/mL in IVF patients. IL-2 levels also showed a slight decline, from approximately 120 pg/mL in healthy individuals to ~115 pg/mL in the IVF group. The absence of statistically significant differences suggests that IVF treatment does not markedly impact Th1 cytokine expression at the systemic level as shown in Figure 1.

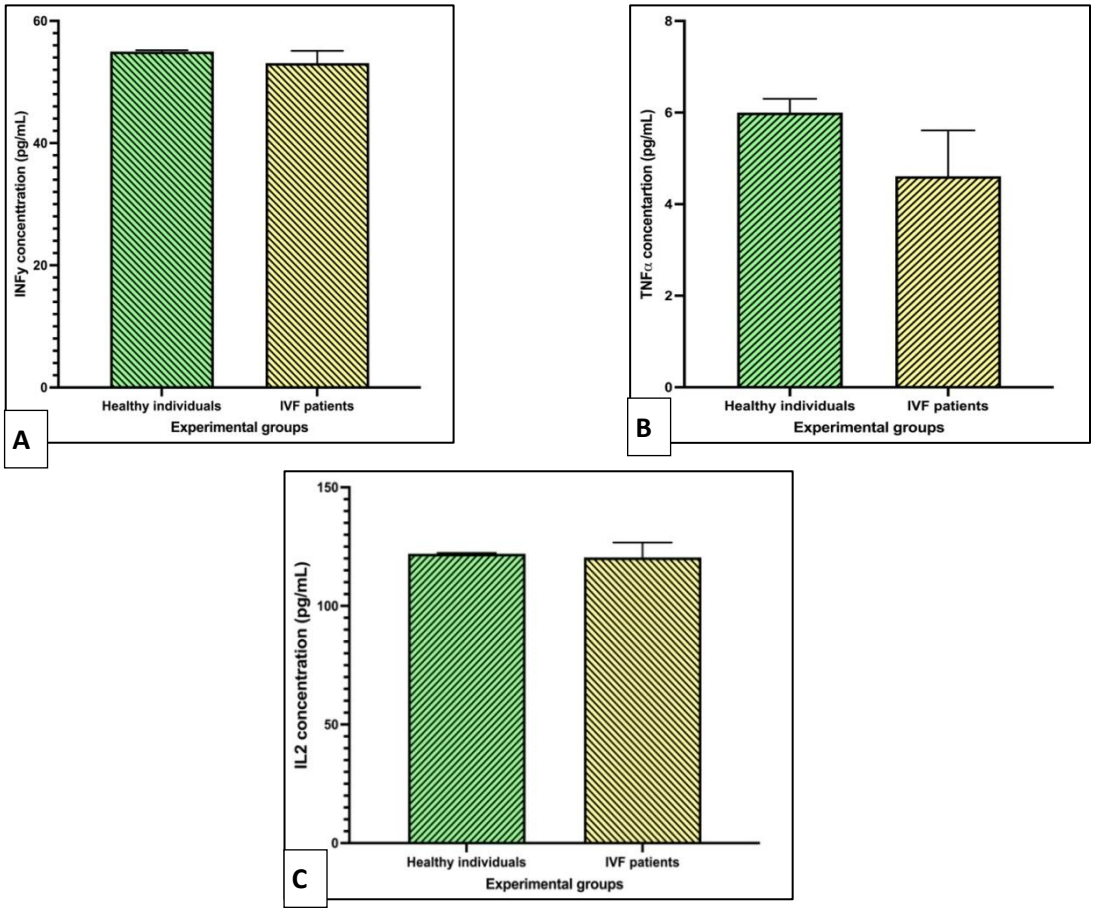

Figure 1. Th1 Cytokines profile expression in healthy and IVF patients

The concentrations of Th1 type cytokines IFN-γ (A), TNF-α (B), and IL-2 (C) in healthy individuals and patients undergoing in vitro fertilization (IVF).

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Statistical comparisons were performed using an unpaired *t*-test. IL-22 concentrations were slightly reduced in IVF patients (~5.2 pg/mL) compared to healthy controls (~6.0 pg/mL); however, this difference was not statistically significant (P >0.05). Similarly, IL-17 levels showed a modest decline from approximately 1.4 pg/mL in healthy individuals to ~1.1 pg/mL in IVF patients, also with no significant difference observed (*P* >0.05). These results indicate that Th17-related cytokine activity, as reflected by IL-17 and IL-22 levels, is not significantly altered in patients undergoing IVF treatment as shown in Figure 2.

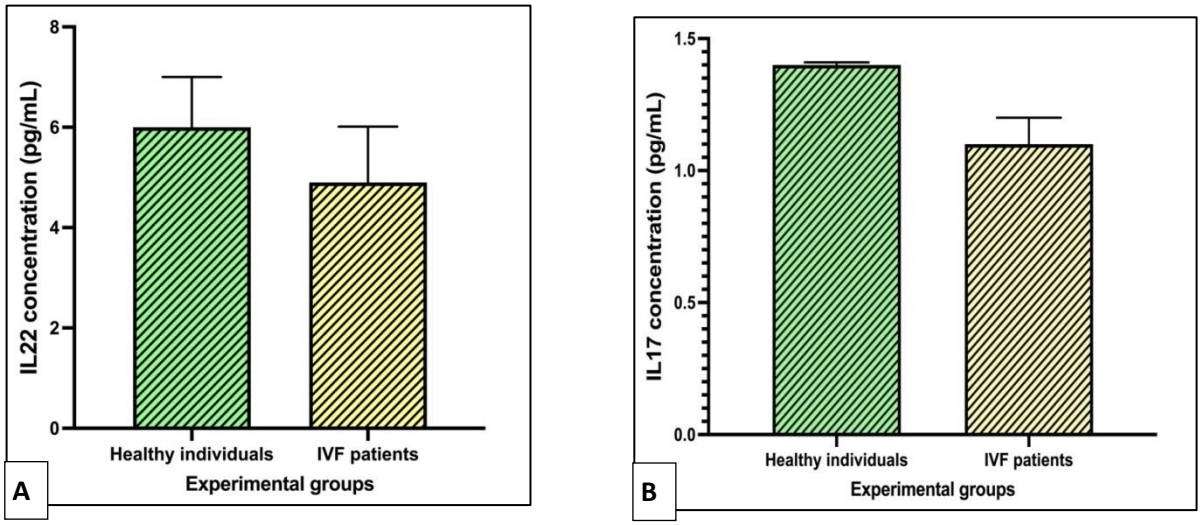

Figure 2. Th17 Cytokines profile expression in healthy and IVF patients

The illustration levels of IL-22 (A) and IL-17 (B) in healthy individuals compared to IVF patients.

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An unpaired *t*-test was used to assess statistical significance. Both cytokines IL-4 and IL-13 were significantly elevated in IVF patients comparedto healthy controls. IL-4 levels increased from approximately 2.0 pg/mL in healthy individuals to ~8.0 pg/mL in IVF patients ($P < 0.01$). Similarly, IL-13 levels rose markedly from around 2.0 pg/mLin controls to ~8.0 pg/mL in IVFpatients ($P < 0.001$). These results indicatea strong upregulation of Th2 cytokine activity in IVF patients, suggesting a shift toward a Th2-dominant immune profile in the IVF context as shown in Figure 3.

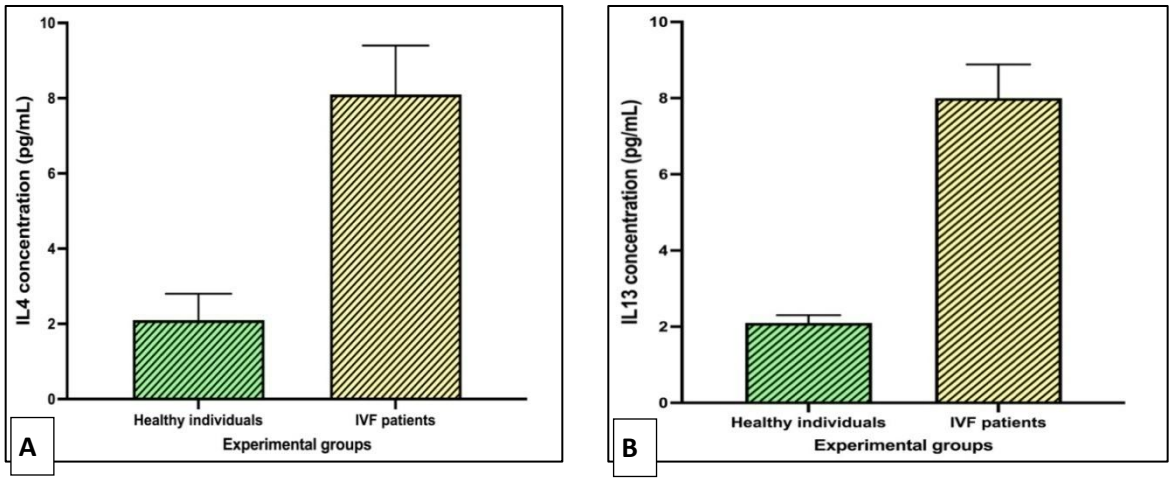

Figure 3. Th2 Cytokines profile expression in healthy and IVF patients

The concentrations of Th2related cytokines IL-4 (A) and IL-13 (B) in healthy individuals and IVF patients. Significant was set as*

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The concentration of IL-10, a key anti-inflammatory cytokine produced by regulatory T (Treg) cells, was significantly elevated in IVF patients compared to healthy individuals. As depicted in the bar graph, IVF patients exhibited a mean IL-10 level of approximately 10 pg/mL, whereas healthy individuals showed a markedly lower mean concentration around 2.5 pg/mL. Statistical analysis revealed a significant difference between the two groups ($P < 0.01$), indicating enhanced Treg activity in the IVF group. This elevation in IL-10 may reflect an immune-modulatory shift toward tolerance, which is critical for successful embryo implantation and pregnancy maintenance in IVF contexts as shown in Figure 4.

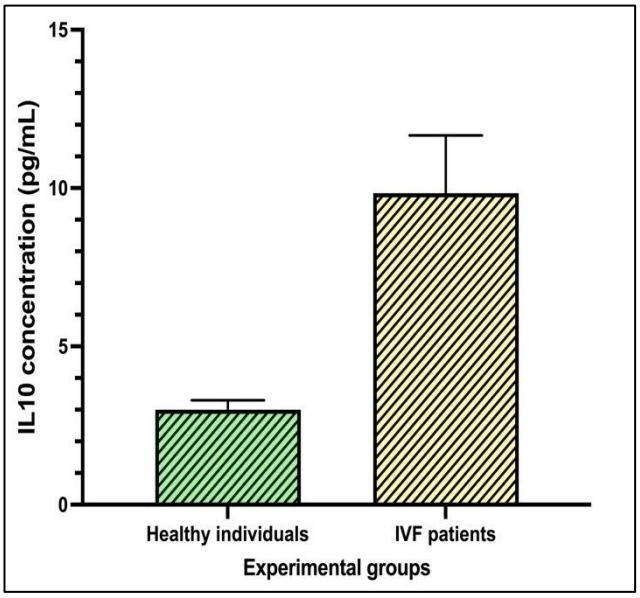

Figure 4. Treg Cytokines profile expression in healthy and IVF patients

The illustration levels of IL-10 (T reg) cell in healthy individuals compared to IVF patients. Significant was set as *

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Discussion

A high prevalence of smoking (57.3%) and prior miscarriage (84.5%) was observed in this study. Predominantly were employed (45.6%) and educated (88.3%). The potential confounding effects of obesity on hormonal measurements were minimised by the fact of mean BMI (24.96) was within the normal range. Nevertheless, the generalisability of the findings to lower income populations may be restricted by the homogeneity of self-perceived economic status (98.1%) (17).The current study investigated the association between reproductive hormones and parity/smoking status among women undergoing fertility evaluation. Our analysis revealed that amongthe variablesassessed,prolactinandAMH(anti-Müllerianhormone)demonstratedstatistically significant differences based on previous pregnancy and smoking status, respectively. Specifically, prolactin levels were significantly higher in women with previous pregnancies compared to those without (P = 0.014), suggesting a potential long-term regulatory role of prolactin in reproductive physiology post-pregnancy. Prolactin is known to modulate the hypothalamic-pituitary-gonadal axis and may have adaptive changes following pregnancy that affect fertility outcomes (18). Moreover, AMH levels were significantly lower in smokers compared to non-smokers(P = 0.027). This finding aligns with prior research indicating that smoking adversely affects ovarian reserve and AMH expression, likely through oxidative stress and follicular atresia mechanisms (19). The AMH reduction among smokers may partly explain reduced reproductive potential observed in this group (35). Other hormones including E2, progesterone, LH, FSH, FT3, FT4, and TSH did not show statistically significant variation with either smoking or previous pregnancy status, consistent with existing studies that report variable hormone sensitivity to lifestyle and reproductive factors (20). These findings underscore the need for personalized reproductive assessments that account for lifestyle and obstetric history, especially considering the modifiable risk factor of smoking on ovarian reserve markers like AMH. Participants with prior IVF attempts displayed elevated LH levels (*P = 0.039), consistent with studies linking higher LH to poorer ovarian response (21). This finding underscores the need for personalized hormonal assessments in IVF protocols to optimize outcomes.

The present study analyzed the Th1 cytokine profile specifically IFN-γ, TNF-α, and IL-2in healthy individuals and patients undergoing in vitro fertilization (IVF). Our findings indicate a modest downregulation of these cytokines in IVF patients compared to healthy controls. IFN-γ, a critical cytokine for

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cellular immunity and implantation success, showed a slight decrease in IVF patients (Figure 1A). This may suggest impaired Th1-type immune activation, which is essential for successful trophoblast invasion and vascular remodelling (22). Consistent with this, previous studies have highlighted that altered IFN-γ expression may negatively influence endometrial receptivity (23). Similarly, TNF-α levels were reduced in IVF patients (Figure 1B). While TNF-α plays a dual role in both promoting and inhibiting implantation depending on the microenvironment and its reduction may reflect an attempt to modulate pro-inflammatory conditions that could otherwise disrupt embryo implantation (24). This aligns with research indicating that controlled TNF-α expression is critical in maintaining immune tolerance during early pregnancy. Interestingly, IL-2 levels, although slightly reduced in IVF patients, remained relatively high (Figure 1C). IL-2 is known to regulate T regulatory cell (Treg) function, which is essential in maternal immune tolerance toward the semi-allogenic fetus (25). The maintenance of near-normal IL-2 levels may represent a compensatory mechanism to sustain immune balance in IVF patients.

Overall, the observed cytokine patterns suggest a subtle but notable shift in Th1-mediated immune responses in IVF patients. This altered immune milieu may partly explain the challenges faced in successful embryo implantation and pregnancy maintenance in these individuals. Future studies with larger cohorts and longitudinal sampling are necessary to validate these trends and explore therapeutic modulation of cytokines to enhance IVF outcomes.

A modest decrease in both IL-22 and IL-17 concentrations in IVF patients compared to healthy individuals. This suggests a potential suppression of Th17-mediated immune responses in the context of IVF procedure or infertility-related immune modulation. IL-22, produced predominantly by Th17 and innate lymphoid cells, plays a critical role in epithelial barrier maintenance and tissue repair (26). The slight reduction of IL-22 in IVF patients (Figure 2A) may reflect compromised mucosal immunity orimpaired endometrial receptivity, both of which could negatively impact embryo implantation. Diminished IL-22 has previously been associated with defective immune-epithelial crosstalk in infertility (27). IL-17, another hallmark Th17 cytokine, is pro-inflammatory and involved in neutrophil recruitment and tissue remodelling.The observed decline in IL-17 levels among IVF patients (Figure 2B) could indicate an immunosuppressive environment or dysregulated inflammatory signalling. While excessive IL-17 activity has been linked to adverse pregnancy outcomes, a complete downregulation might hinder necessary local immune activation during implantation (28).

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These findings align with reports suggesting that balanced IL-17 signalling is essential for successful embryo implantationand placental development. Collectively, the data suggest that IVF patients may exhibit impaired Th17 cytokine activity, which could contribute to suboptimal endometrial conditions. These findings underline the importance of Th17-associated immune regulation in reproductive success and suggest a potential avenue for therapeutic intervention.

The significant elevation suggests a shift toward a Th2-dominant immune profile, which may influence the immune-endocrine balance critical for implantation and early pregnancy maintenance. IL-4, a prototypical Th2 cytokine, was substantially elevated in IVF patients (Figure 3A). IL-4 is known to promote humoral immunity and inhibit Th1 responses, contributing to an anti-inflammatory environment within the endometrium (29). Although a Th2-dominant profile has been considered beneficial for pregnancy maintenance, excessive Th2 polarization might impair the necessary immune activation required for embryo implantation (30). Thus, the observed IL-4 increase may reflect an overcompensation that hinders rather than supports successful implantation. Similarly, IL-13 levels were significantly higher in IVF patients (Figure 3B). Like IL-4, IL-13 is involved in the regulation of allergic and anti-inflammatory responses and can inhibit macrophage activation and antigen presentation (31). The increased IL-13 concentration may further skew the immune environment towards a tolerogenic state, potentially at the expense of appropriate local immune activation necessary for embryo recognition and invasion. These findings are consistent with previous reports indicating that altered Th1/Th2 balance, particularly exaggerated Th2 responses, is associated with implantation failure and recurrent IVF failure (32). While immune tolerance is crucial for maternal-fetal coexistence, optimal implantation appears to require a fine-tuned equilibrium between inflammatory and regulatory cytokines.

The results demonstrated a significantly higher concentration of IL-10 in IVF patients compared to healthyindividuals,suggesting an upregulation of Treg cell-mediated immunosuppression during IVF. IL-10 plays a critical role in promoting maternal-fetal immune tolerance by inhibiting pro-inflammatory responses and supporting embryo implantation and pregnancy continuation (33). The increased IL-10 levels observed in IVF patients may reflect a compensatory mechanism to counterbalance immune activation induced by ovarian stimulation and embryo transfer procedures. These findings are consistent with recent studies indicating that successful IVF outcomes are

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associated with a shift toward an anti-inflammatory, Treg-dominant immune profile (34). Enhancing IL-10-mediated pathwaysmay thus represent a therapeutic strategy to improve implantation rates and reduce the risk of pregnancy loss in assisted reproduction. This study has some limitations that should be taken in consideration while interpretating the outcomes. Due to the small number of fertility centres from which the study samples were drawn, selection bias may have been introduced, hence reducing external validity. Numerous circumstances, such as stress, sickness or periods that may not have been properly regulated could influence cytokines levels (36). It is advised that these findings be confirmed and expanded upon in future research using bigger, multicentre cohorts and more comprehensive immunological profile.

Conclusion

The interaction between Th1 (IFN-γ, TNF-α, IL-2), Th2 (IL-4, IL-13), Th17 (IL-17, IL-22), and Treg (IL-10) cells is key to the immune tolerance required for successful IVF outcomes. Implantation and pregnancy maintenance are supported by a shift towards Th2 and Treg cell activity, whereas IVF failure is associated with the dominance of Th1 and Th17 response. Understanding these immune patterns not only sheds light on the causes of reproductive failure but also paves the way for therapies aimed at improving IVF success rates. Future studies should evaluate whether modulating Th2 cytokine levels either pharmacologically or via immunotherapy can improve implantation success in IVF patients. Additionally, longitudinal cytokine profiling during different IVF phases could help in developing personalized therapeutic strategies.

Competing interest

The authors declare that they have no competing interests.

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