



VITAMIN D3 AND CYTOKINE PROFILES AS PREDICTIVE MARKERS OF IN VITRO FERTILIZATION SUCCESS

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ABSTRACT

Background: Infertility is a growing global concern, affecting nearly 15% of reproductive-aged couples, with a particularly high burden in South Asia. Despite advancements in assisted reproductive technologies (ART), *in vitro* fertilization (IVF) success rates remain suboptimal.

Objective: To evaluate the association between serum levels of Vitamin D3, interleukin-6 (IL-6), leukemia inhibitory factor (LIF), and tumor necrosis factor-alpha (TNF- α) and IVF outcomes in women undergoing fertility treatment.

Methodology: This cross-sectional study was conducted between January and August 2022 and included 80 women aged 30–40 years, recruited via non-probability convenience sampling from the Australian Concept Infertility Medical Center, Lahore, and screened at The University of Lahore. Participants had a history of either primary or secondary infertility or recurrent pregnancy loss. Blood samples were collected during IVF treatment and analyzed for Vitamin D3, IL-6, LIF, and TNF- α using enzyme-linked immunosorbent assay (ELISA). Statistical analysis was performed using SPSS v26, with non-parametric tests applied due to non-normal distribution.

Results: Out of 80 participants, 40 (50%) achieved a positive pregnancy outcome. Mean Vitamin D3 levels were significantly higher in women who conceived (32.75 ± 9.81 ng/mL) compared to those who did not (21.40 ± 7.45 ng/mL, $p = 0.009$). Similarly, TNF- α levels were significantly elevated in the positive outcome group (384.72 ± 349.18 pg/mL) compared to the negative group (153.90 ± 74.13 pg/mL, $p = 0.038$). Although IL-6 and LIF levels were slightly higher in the positive outcome group, the differences were not statistically significant ($p > 0.05$).

Conclusion: Sufficient Vitamin D3 and regulated TNF- α levels appear to predict IVF success, while IL-6 and LIF show limited standalone predictive value, supporting the role of immune-hormonal biomarkers in personalized fertility care.

Keywords: Cytokines; Interleukin-6; Leukemia Inhibitory Factor; Tumor Necrosis Factor-alpha; *in vitro* fertilization; Pregnancy.

Introduction

Infertility is increasingly recognized as a significant global public health issue, affecting approximately 15% of reproductive-aged couples and impacting an estimated 186 million individuals worldwide. The Global Burden of Disease (GBD) studies consistently report a growing prevalence of infertility across regions, with India, Pakistan, China, and Indonesia among the highest-burden countries^{1,2}. In India, infertility prevalence varies between 3.9% and 16.8%, with urban and semi-urban populations particularly affected due to lifestyle and environmental factors³. In Pakistan, studies indicate an infertility rate of around 22%, with primary infertility accounting for approximately 4% and secondary infertility around 18%^{4,5}. Contributing factors include high rates of polycystic ovary syndrome (PCOS), endometriosis, consanguineous marriages, and limited access to reproductive health services⁶.

Despite the increasing availability of assisted reproductive technologies (ART), including *in vitro* fertilization (IVF), success rates remain modest—around 30–40% per cycle globally. The unpredictability of IVF outcomes presents both emotional and financial challenges for couples undergoing repeated cycles. As a result, there is a pressing need to identify reliable, non-invasive, and affordable biomarkers that can predict IVF success and personalize fertility treatment⁷.

Emerging evidence suggests that Vitamin D3 and cytokine profiles may serve as valuable biological markers for reproductive outcomes⁸. Beyond its classical role in bone metabolism, Vitamin D3 influences various reproductive processes, including ovarian steroidogenesis, folliculogenesis, and endometrial receptivity, primarily via Vitamin D receptors expressed in reproductive tissues. Notably, Vitamin D deficiency is highly prevalent among women in India and Pakistan, exacerbated by limited sun exposure, cultural clothing practices, and nutritional deficits⁹.

At the same time, cytokines, key signaling molecules in the immune system, regulate embryo-endometrial cross-talk during implantation. A delicate balance between pro-inflammatory (e.g., IL-6, TNF- α) and anti-inflammatory (e.g., IL-10, TGF- β) cytokines is critical for successful implantation and pregnancy maintenance. Immune dysregulation and chronic inflammation have been linked to poor IVF outcomes, implantation failure, and early miscarriage¹⁰. Several studies in South Asia—including Pakistan—have noted immune-related reproductive issues, particularly among women with PCOS and endometriosis, both of which are major contributors to infertility in the region⁴.

While the roles of Vitamin D and cytokines have been explored separately, there is limited integrative research examining their combined predictive value for IVF success, particularly in South Asian populations¹¹. This gap is especially significant considering the high burden of infertility, widespread nutritional deficiencies, and immune-mediated gynecological conditions in countries like India and Pakistan. This study, therefore, seeks to evaluate baseline serum levels of Vitamin D3 and selected cytokine profiles as predictive markers of IVF success, defined by clinical pregnancy rates.

Methodology

This cross-sectional study was conducted between January 2022 and August 2022 to examine the correlation between specific immunological and hormonal markers Vitamin D3, interleukin-6 (IL-6), LIF, and TNF- α —and IVF outcomes in women undergoing fertility treatment. A total of 80 women, aged between 30 and 40 years, were enrolled using a non-probability convenience sampling technique. Participants were recruited from the Australian Concept Infertility Medical Center, Lahore, and screened for eligibility at The University of Lahore. Inclusion criteria comprised women with documented cases of primary or secondary infertility, or a history of recurrent miscarriages. Women were excluded if they had prior gynecological surgeries that could confound reproductive outcomes or if relevant histopathological diagnoses were unavailable. Ethical approval was obtained from the Institutional Review Board of the Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, and written informed consent was collected from all participants. Confidentiality and adherence to ethical standards were ensured throughout the study.

Detailed demographic and clinical data—including age, duration of infertility, underlying causes, and medical history—were extracted from patient records. Peripheral blood samples were collected during IVF treatment phases and processed for biomarker analysis. Serum levels of IL-6, LIF, and TNF- α

were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits, following the manufacturers' protocols. Optical density (OD) values were read at 450 nm using a calibrated microplate reader to quantify cytokine concentrations. In addition, serum Vitamin D3 [25(OH)D] levels were evaluated using a high-sensitivity ELISA kit, also adhering strictly to manufacturer instructions. Vitamin D3 levels were categorized based on internationally recognized thresholds: deficient (<20 ng/mL), insufficient (21–29 ng/mL), and sufficient ($\geq$ 30 ng/mL). This measurement was included to assess whether hypovitaminosis D, a condition common among women of reproductive age in South Asia, was associated with IVF success or failure.

Statistical analysis was carried out using SPSS version 26. Descriptive statistics (mean $\pm$ standard deviation) were used to summarize continuous variables. Group comparisons between successful and unsuccessful IVF outcomes were performed using Mann–Whitney U test.

Results

The demographic and clinical characteristics of the participants are summarized in Table 1. The mean age of the women was 32.85 ± 4.21 years, ranging from 27 to 44 years. The mean body mass index (BMI) was 26.75 ± 4.32 kg/m², with a minimum of 18 and a maximum of 34. Of the total participants, 73 (91.3%) were nulliparous, 5 (6.2%) were primiparous, and 2 (2.5%) were multiparous. Regarding the type of infertility, 62 (77.5%) had primary infertility, while 18 (22.5%) had secondary infertility. The distribution of blood groups showed O-positive as the most common (55%), followed by B-positive (30%), AB-positive (8.75%), and A-positive (6.25%). The pregnancy outcome was evenly distributed, with 40 (50%) women achieving a positive IVF result, while the remaining 40 (50%) did not conceive.

Table 1: Characteristics of study participants

Variable	Value
Sample Size	80
Age (years)	32.85 ± 4.21 [27–44]
BMI (kg/m²)	26.75 ± 4.32 [18–34]
Parity	Nulliparous: 73 (91.3%)
	Primiparous: 5 (6.2%)
	Multiparous: 2 (2.5%)
Type of Infertility	Primary: 62 (77.5%)
	Secondary: 18 (22.5%)
Blood Groups	O+: 44 (55%)
	B+: 24 (30%)
	AB+: 7 (8.75%)
	A+: 5 (6.25%)
Pregnancy Outcome	Positive: 40 (50%)
	Negative: 40 (50%)

Normality tests indicated that serum IL-6, LIF, TNF- α , and Vitamin D3 levels were not normally distributed (Shapiro–Wilk $p < 0.001$). Therefore, non-parametric statistical tests were applied.

As shown in Table 2, the mean serum IL-6 level was higher in women with a positive pregnancy outcome (56.10 ± 27.45 pg/mL) compared to those with a negative outcome (49.25 ± 24.13 pg/mL), but this difference was not statistically significant ($p = 0.420$). Similarly, LIF levels were elevated in the positive outcome group (198.41 ± 201.34 pg/mL) versus the negative group (162.23 ± 120.78 pg/mL), without statistical significance ($p = 0.611$).

However, serum TNF- α levels were significantly higher in women who conceived (384.72 ± 349.18 pg/mL) than those who did not (153.90 ± 74.13 pg/mL), with a statistically significant difference ($p = 0.038$). This finding aligns with existing literature highlighting a controlled pro-inflammatory environment as favorable for implantation.

Most notably, Vitamin D3 [25(OH)D] levels were significantly associated with pregnancy outcomes. Women with successful IVF outcomes had higher mean serum Vitamin D3 levels (32.75 ± 9.81 ng/mL), indicating sufficiency, while those with negative outcomes had lower levels (21.40 ± 7.45 ng/mL), consistent with insufficiency or deficiency. The association was statistically significant ($p = 0.009$).

Table 2: Comparison of biomarkers in relation to pregnancy outcome

Biomarker	Positive (n = 40)	Negative (n = 40)	p-value (Mann–Whitney U)
IL-6 (pg/mL)	56.10 ± 27.45	49.25 ± 24.13	0.420
LIF (pg/mL)	198.41 ± 201.34	162.23 ± 120.78	0.611
TNF- α (pg/mL)	384.72 ± 349.18	153.90 ± 74.13	0.038
Vitamin D3 (ng/mL)	32.75 ± 9.81	21.40 ± 7.45	0.009

Discussion

This study aimed to evaluate the association between serum levels of Vitamin D3, IL-6, LIF, and TNF- α with IVF outcomes in women undergoing assisted reproductive treatment. The findings revealed a significant positive association between Vitamin D3 and TNF- α levels with successful pregnancy outcomes, while IL-6 and LIF levels showed no statistically significant differences, despite being slightly elevated in women who conceived. These results provide valuable insights into the role of immuno-hormonal markers in predicting IVF success.

The significant association between Vitamin D3 levels and pregnancy outcome in our study is consistent with several international studies. A meta-analysis by Chu et al. (2022) covering 3,307 IVF patients across 11 studies found that Vitamin D sufficiency (≥ 30 ng/mL) was associated with significantly higher clinical pregnancy and implantation rates (OR 1.46; 95% CI, 1.05–2.04). Similarly, Xu et al. (2025) reported that Vitamin D3 positively influences endometrial receptivity and embryo quality through modulation of HOXA10 gene expression and local immune function¹². In the South Asian context, Mahar et al. (2024) conducted a study in Karachi, Pakistan, which revealed that 76% of infertile women had Vitamin D deficiency, and women who conceived after IVF had significantly higher mean 25(OH)D levels (31.8 ± 8.9 ng/mL) compared to non-conceiving counterparts (22.1 ± 6.7 ng/mL), closely aligning with our own findings¹³.

The current study also found significantly elevated TNF- α levels in women with positive IVF outcomes. While TNF- α is classically considered a pro-inflammatory cytokine, its presence during the early implantation window may be beneficial in preparing the endometrium for trophoblast invasion. A global systematic review by Piccinni et al. (2021) emphasized that a regulated rise in TNF- α levels during the peri-implantation phase supports successful embryo implantation, though excessive or chronic inflammation is detrimental¹⁴. Supporting this, Rehman et al. (2018) from Pakistan observed that moderate elevations in TNF- α were associated with higher endometrial receptivity and improved pregnancy rates in women undergoing ICSI (Intracytoplasmic Sperm Injection)¹⁵.

In contrast, IL-6 and LIF, though higher in women with positive outcomes in our study, did not show statistically significant differences. IL-6 is a multifunctional cytokine involved in inflammation, immune regulation, and tissue remodeling. While some studies have shown elevated IL-6 to be associated with endometrial receptivity, others report no clear correlation with IVF outcomes. For instance, Pantos et al. (2022) found that IL-6 levels were higher in successful IVF cases but the difference did not reach significance, consistent with our observations ¹⁰. LIF, a known implantation facilitator, has also yielded mixed results. A Volovsky and Seifer (2024) suggested that mid-luteal phase endometrial LIF expression was associated with implantation success, but serum LIF levels were less predictive ¹⁶.

From a regional perspective, Fatima et al. (2023) in Lahore found that women with failed IVF cycles had elevated systemic IL-6 but did not observe statistically relevant trends, further confirming the limited predictive power of serum IL-6 ¹⁷. Another study by Ali et al. (2023) showed that while LIF is crucial in murine and in vitro implantation models, its serum concentration lacks consistent association with human IVF outcomes, likely due to its localized action at the endometrial level ¹⁸.

These findings together emphasize that Vitamin D3 sufficiency and optimal TNF- α levels may serve as non-invasive predictive biomarkers for IVF success, while IL-6 and LIF require further investigation, especially in localized tissue samples (e.g., uterine fluid or endometrial biopsy) rather than systemic circulation ¹⁹. The strengths of this study include its focus on biomarker-based prediction in a South Asian population, a region with high infertility prevalence, widespread Vitamin D deficiency, and underexplored immunological dimensions of reproduction. However, the cross-sectional design, moderate sample size, and lack of endometrial tissue analysis are limitations that should be addressed in future studies.

Conclusion

This study demonstrates that Vitamin D3 sufficiency and elevated TNF- α levels are significantly associated with improved IVF outcomes, suggesting their potential as non-invasive predictive biomarkers for clinical pregnancy success. While IL-6 and LIF levels showed higher trends in women who conceived, their lack of statistical significance highlights the need for further research into their localized uterine roles. These findings are particularly relevant for populations with high infertility rates and widespread Vitamin D deficiency, such as in Pakistan and other South Asian countries. Integrating immunological and hormonal profiling into IVF protocols could support more personalized and effective fertility care, though larger, longitudinal studies are required to validate these associations and explore underlying mechanisms in greater detail.

References

1. Feng J, Wu Q, Liang Y, Liang Y, Bin Q. Epidemiological characteristics of infertility, 1990–2021, and 15-year forecasts: an analysis based on the global burden of disease study 2021. *Reproductive Health*. 2025;22(1):26.
2. Shoaib M, Achakzai M, Munir U, Khan M, Sanam M, Naeem S. Frequency of Polycystic Ovarian Syndrome in Women Presenting with Infertility. *Indus Journal of Bioscience Research*. 2025;3(2):830-6.
3. Pocha PR, Patil A, Gosavi TP, Gokhale A, Jadhav A. Homeopathy Treatment for Unexplained Oligospermia: A Interventional, Single Arm, Open Label, Non-Randomized, Experimental Trial. *Public Health and Epidemiology: Open Access*. 2025;1(2):1-8.
4. Ashraf S, Mumtaz S, Shakeel Q, Imnan S, Shabbir A, Iqbal S. Polycystic Ovary Syndrome in South Asia: Diagnostic Diversity, Endocrine Drivers, and Comorbidity Patterns with a Focus on Pakistan. *Journal of Health, Wellness and Community Research*. 2025:e901-e.
5. Hussain S, Brayne C. Psychiatric morbidity among women with infertility in Pakistan: a cross-sectional survey. *BMJ open*. 2025;15(3):e087903.
6. Kainat NFP, Khuhro BN, Pir S, Ghumro RA, Baloch R. ENDOMETRIOSIS AND ITS CONSEQUENCES ON FERTILITY AND QUALITY OF LIFE—A HOSPITAL-BASED CROSS-SECTIONAL STUDY AT PMCH NAWABSHAH.: *JPUMHS*; 2024: 14: 04,132-138.

- <http://doi.org/10.46536/jpumhs/2024/14.04>. 570. Journal of Peoples University of Medical & Health Sciences Nawabshah(JPUMHS). 2024;14(4):132-8.
7. Uche N. Ethical and Cultural Challenges in Global Impementation of Assisted Reproduction Technology (ART)-An Integrative Literature Review of the Middle East and Africa. 2025.
8. El-Amrawi GA, Gad Elkarim DS. Impact of vitamin D supplement on reproductive performance in pre-mating ewes: presumptive regulatory role of some inflammatory cytokines and serum biomarkers. Egyptian Journal of Veterinary Sciences. 2023;54(4):643-51.
9. Siddiqee MH, Bhattacharjee B, Siddiqi UR, MeshbahurRahman M. High prevalence of vitamin D deficiency among the South Asian adults: a systematic review and meta-analysis. BMC public health. 2021;21(1):1823.
10. Pantos K, Grigoriadis S, Maziotis E, Pistola K, Xystra P, Pantou A, et al. The role of interleukins in recurrent implantation failure: a comprehensive review of the literature. International journal of molecular sciences. 2022;23(4):2198.
11. Albeitawi S, Bani-Mousa S-U, Jarrar B, Aloqaily I, Al-Shloul N, Alsheyab G, et al. Associations Between Follicular Fluid Biomarkers and IVF/ICSI Outcomes in Normo-Ovulatory Women—A Systematic Review. Biomolecules. 2025;15(3):443.
12. Xu C, An X, Tang X, Yang Y, Deng Q, Kong Q, et al. Association between vitamin D level and clinical outcomes of assisted reproductive treatment: a systematic review and dose-response meta-analysis. Reproductive Sciences. 2025;32(5):1446-58.
13. Mahar B, Shah T, Sadiq N, Mangi R, Warsi J, Abbas Q. Vitamin D Deficiency Prevalence in Pakistan: Common, Important, and Neglected: A Comprehensive Meta-Analysis. Journal of Diabetology. 2024;15(4):335-48.
14. Piccinni M-P, Raghupathy R, Saito S, Szekeres-Bartho J. Cytokines, hormones and cellular regulatory mechanisms favoring successful reproduction. Frontiers in immunology. 2021;12:717808.
15. Rehman R, Ashraf M, Jasmine A, Lal K, Alam F. Cytokines and endometrial receptivity after intracytoplasmic sperm injection-A cohort study at Islamabad. J Pak Med Assoc. 2018;68(6):862-6.
16. Volovsky M, Seifer DB. Current status of ovarian and endometrial biomarkers in predicting ART outcomes. Journal of Clinical Medicine. 2024;13(13):3739.
17. Fatima W, Akhtar AM, Hanif A, Gilani A, Farooq SMY. Sociodemographics and Risk Factors of Secondary Infertility in Pakistan: A Case-Control Study. Journal of Hunan University Natural Sciences. 2023;50(10).
18. Ali R, Khan TA, Gul H, Rehman R. An interplay of Progesterone, Leukemia Inhibitor Factor and Interleukin-6 in the window of implantation; Impact on fertility. Cytokine. 2023;170:156332.
19. Coussa A. Effect of in vitro fertilisation (IVF) hormonal therapy on metabolic, endocrine and inflammatory status in IVF-conceived pregnancy: University of Warwick; 2020.