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Follicular fluid cytokine and homocysteine profiles in poor ovarian responders with and without sonographic endometrioma: A comparative study

Sonografik endometrioması olan ve olmayan düşük over rezervli kadınlarda foliküler sıvı sitokin ve homosistein profilleri: Karşılaştırmalı çalışma

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Abstract

Objective: To compare follicular fluid (FF) cytokine and homocysteine profiles in women with poor ovarian response (POR) undergoing in vitro fertilization (IVF), with and without sonographic endometrioma, and to explore potential inflammatory alterations associated with endometrioma in this population.

Materials and Methods: This prospective comparative study was conducted among 60 women diagnosed with POR who were undergoing IVF treatment. Participants were divided into two groups according to the presence of sonographic endometrioma: Group I included women without sonographic endometrioma (n=30) and Group II included women with sonographic endometrioma (n=30). FF samples were collected during oocyte retrieval and analyzed for inflammatory biomarkers. Concentrations of interleukin-1β (IL-1β), IL-6, IL-8, IL-10, IL-12p70, IL-17A, IL-18, IL-23, IL-33, interferon-α2 (IFN-α2), IFN-γ, tumor necrosis factor-α (TNF-α), monocyte chemoattractant protein-1 (MCP-1), and homocysteine were measured using LEGENDplex multiplex assays and flow cytometry. Cytokine and homocysteine levels were compared between groups.

Results: Most inflammatory cytokines, including IL-1β, IL-6, IL-8, IFN-γ, and MCP-1, showed lower levels in women with sonographic endometrioma compared with women without sonographic endometrioma. In contrast, TNF-α and IL-33 levels tended to be higher in the endometrioma group. Homocysteine levels were also lower in women with sonographic endometriomas. However, none of the observed differences reached statistical significance. Overall, the findings suggested distinct, albeit non-significant, inflammatory trends in the FF microenvironment of women with POR and sonographic endometrioma.

Conclusion: Women with POR and sonographic endometrioma showed altered trends in FF inflammatory-marker profiles compared with women without sonographic endometrioma; however, these differences were not statistically significant. Since the absence of sonographic endometrioma does not exclude endometriosis, the findings should be interpreted cautiously. Larger prospective studies that include IVF and assess embryological and reproductive outcomes are required to clarify the clinical significance of FF biomarkers in women with POR and endometrioma.

Keywords: Endometrioma, poor ovarian response, follicular fluid, cytokines, homocysteine, IVF

PRECIS: Women with poor ovarian response and sonographic endometrioma showed directional but non-significant differences in selected follicular fluid cytokines. The findings are exploratory and require validation using in vitro fertilization outcomes.

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Öz

Amaç: Bu çalışmanın amacı, in vitro fertilizasyon (IVF) tedavisi gören düşük over rezerv (DOR) tanılı kadınlarda, sonografik endometrioma varlığına göre follikül sıvısı (FS) sitokin ve homosistein profillerini karşılaştırmak ve endometrioma ile ilişkili olası enflamatuvar değişiklikleri araştırmaktır.

Gereç ve Yöntemler: Bu prospektif karşılaştırmalı çalışmaya IVF tedavisi gören ve DOR tanısı bulunan toplam 60 kadın dahil edildi. Katılımcılar sonografik endometrioma varlığına göre iki gruba ayrıldı: Grup I, sonografik endometrioması olmayan kadınlardan (n=30); Grup II ise sonografik endometrioması bulunan kadınlardan (n=30) oluştu. FS örnekleri oosit toplama işlemi sırasında elde edildi ve inflamatuvar biyobelirteçler açısından analiz edildi. İnterlökin-1β (IL-1β), IL-6, IL-8, IL-10, IL-12p70, IL-17A, IL-18, IL-23, IL-33, interferon-α2 (IFN-α2), IFN-γ, tümör nekroz faktörü-α (TNF-α), monosit kemoatraktan protein-1 (MCP-1) ve homosistein düzeyleri LEGENDplex multipleks analiz yöntemi ve akım sitometrisi kullanılarak ölçüldü. Sitokin ve homosistein düzeyleri gruplar arasında karşılaştırıldı.

Bulgular: IL-1β, IL-6, IL-8, IFN-γ ve MCP-1 dahil olmak üzere çoğu enflamatuvar sitokin düzeyi, sonografik endometrioması bulunan kadınlarda, sonografik endometrioması olmayan kadınlara kıyasla daha düşük bulundu. Buna karşılık TNF-α ve IL-33 düzeyleri endometrioma grubunda daha yüksek eğilim gösterdi. Homosistein düzeyleri de sonografik endometrioması bulunan kadınlarda daha düşük saptandı. Ancak gözlenen farklılıkların hiçbiri istatistiksel anlamlılığa ulaşmadı. Genel olarak bulgular, DOR ve sonografik endometrioması bulunan kadınların FS mikroçevresinde belirgin ancak istatistiksel olarak anlamlı olmayan inflamatuvar eğilimler olduğunu düşündürdü.

Sonuç: DOR ve sonografik endometrioması bulunan kadınlarda, sonografik endometrioması olmayan kadınlara kıyasla FS enflamatuvar belirteç profillerinde değişmiş eğilimler gözlenmiş, ancak istatistiksel olarak anlamlı farklılık saptanmamıştır. Sonografik endometrioma yokluğunun endometriozisi dışlamadığı göz önünde bulundurularak bulgular dikkatli yorumlanmalıdır. IVF, embriyolojik ve üreme sonuçlarını içeren daha geniş prospektif çalışmalara, DOR ve endometriomalı kadınlarda FS biyobelirteçlerinin klinik önemini daha iyi açıklığa kavuşturmak için ihtiyaç vardır.

Anahtar Kelimeler: Endometrioma, düşük over rezervi, foliküler sıvı, sitokinler, homosistein, IVF

Introduction

Endometriosis is a chronic inflammatory condition characterized by the presence of endometrial-like tissue outside the uterus, affecting approximately 10-15% of women of reproductive age and up to 40% of infertile women[1,2]. Despite significant advances in diagnostic imaging and surgical treatment, the pathophysiology of endometriosis remains complex and multifactorial, involving hormonal, immunological, and genetic factors[3-5].

Several immunological abnormalities have been implicated in the development and progression of endometriosis, including altered macrophage activity, cytokine imbalance, and impaired natural killer cell function[6-8]. The local peritoneal and follicular environment in affected individuals is often enriched with pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), IL-8, and monocyte chemoattractant protein-1 (MCP-1), which may contribute to abnormal folliculogenesis, impaired oocyte quality, and reduced implantation potential[9-13].

The follicular fluid (FF) is a key microenvironment supporting oocyte development and maturation. It contains a wide array of soluble factors—cytokines, growth factors, metabolites—that mediate paracrine signaling and reflect both systemic and local ovarian conditions[14]. In women with endometriosis, the FF may exhibit a disrupted immunological milieu, potentially compromising oocyte competence and embryo development during in vitro fertilization (IVF)[15].

Poor ovarian response (POR) to controlled ovarian stimulation, defined by the Bologna criteria or more recently the POSEIDON classification, presents an additional challenge in assisted reproductive technologies[16,17]. Women with POR often show altered inflammatory and metabolic signaling in FF, with higher levels of oxidative stress markers and reduced concentrations of growth-promoting cytokines[18]. The coexistence of POR and endometriosis may further exacerbate this unfavorable follicular environment, although few studies have examined this specific subgroup in detail.

Recent studies suggest that evaluating FF cytokines and metabolic markers such as homocysteine may offer insight into the pathophysiology of oocyte competence, particularly in complex infertility cases[19,20]. Homocysteine, a sulfur-containing amino acid involved in methylation pathways, has been linked to impaired follicular angiogenesis, mitochondrial dysfunction, and increased oxidative stress, all of which may affect oocyte and embryo quality[21].

The present study aims to compare the cytokine and homocysteine profiles in FF from women with POR, both with and without sonographically confirmed endometrioma. By focusing on this underexplored intersection, we hope to identify immunological or metabolic differences that may contribute to reduced fertility outcomes and may help generate hypotheses for individualized strategies in IVF.

Materials and Methods

Study Design and Participants

This prospective comparative study was conducted at the Assisted Reproductive Technologies Unit of Acıbadem Maslak Hospital, İstanbul, Türkiye, as an exploratory biomarker analysis in women with POR comparing patients with and without sonographic endometrioma. No single primary biomarker was predefined; a predefined inflammatory marker panel and homocysteine levels were evaluated to generate hypotheses. A total of 60 infertile women diagnosed with POR were recruited and divided into two groups: those

with a sonographically confirmed diagnosis of endometrioma (n=30) and those without (n=30) (Figure 1). This was a pilot exploratory study. No a priori sample size calculation was performed. The target sample size of 30 per group was determined based on feasibility within the study period. All participants had a history of infertility of at least one year and at least one functional ovary.

Inclusion criteria followed the POSEIDON classification [Groups 3 and 4: antral follicle count (AFC) <5 and anti-Müllerian hormone (AMH) <1.2 ng/mL, stratified by age] [17] . Exclusion criteria included severe male factor infertility (azoospermia, cryptozoospermia), congenital or acquired uterine anomalies, polycystic ovary syndrome, recurrent pregnancy loss, and recurrent implantation failure.

Endometriosis Characterization: In the endometrioma group, endometriomas were identified using standard transvaginal ultrasound criteria (thick-walled, homogeneous, low-level internal echoes). Where available, endometrioma size (maximum diameter, mm) and laterality (unilateral/bilateral) were abstracted from clinical records. Deep infiltrating endometriosis was not systematically assessed. Prior endometriosis surgery and medical/hormonal treatments were recorded where available. The duration of infertility (months) was recorded at enrollment. All assessments were performed by experienced clinicians at the beginning of the ovarian stimulation cycle.

Ovarian Stimulation Protocol and Oocyte Retrieval

All participants underwent a controlled ovarian hyperstimulation protocol. Stimulation was initiated on days 2-4 of the menstrual cycle using recombinant follicle-stimulating hormone (FSH; 150-300 IU daily), with or without the addition of human menopausal gonadotropin, based on clinical judgment. Serial transvaginal ultrasonography and serum estradiol (E2) measurements guided dose adjustments. Final oocyte maturation was triggered with 6500 IU human chorionic gonadotropin in combination with 0.2 mg gonadotropin-releasing hormone agonist when at least one follicle reached ≥18 mm or three follicles were ≥17 mm in diameter. Oocyte retrieval was performed 36 hours post-trigger under sedation.

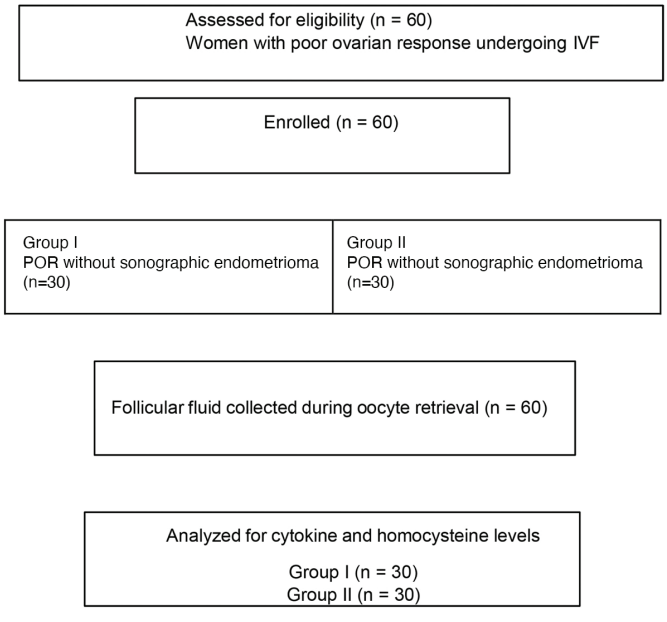

Figure 1. The flowchart of the study

IVF: In vitro fertilization, POR: Poor ovarian response

FF Collection and Analysis

Immediately following oocyte retrieval, FF was aspirated from the first accessible ≥18 mm follicle prior to any flushing. When multiple mature follicles were present, only the first aspirated follicle was used; follicles were not pooled. Tubes were inspected immediately; samples with visible blood contamination (reddish discoloration, hemolysis) were discarded. Cumulus-oocyte complexes were separated, and the remaining FF was centrifuged at 450 g for 5 minutes at room temperature. The supernatant was aliquoted and stored at -20 °C within 60 minutes of retrieval; a single freeze-thaw cycle was permitted for analysis, and no aliquot underwent more than one cycle. Assays were performed at the Acıbadem Labmed Clinical Laboratory using the LEGENDplex Human Inflammation Panel 1 (BioLegend, Germany), a bead-based multiplex flow cytometry assay, on a BD FACSCanto II flow cytometer. Standard curves and controls were run on each plate. Analysts and laboratory personnel were blinded to clinical data and group assignment. Concentrations of IL-1β, IL-6, IL-8, IL-10, IL-12p70, IL-17A, IL-18, IL-23, IL-33, IFN-α2, IFN-γ, TNF-α, MCP-1, and homocysteine were measured and analyzed with LEGENDplex Data Analysis Software.

Ethical Approval

The study protocol was approved by the Institutional Review Board and the Ethics Committee of Acıbadem University (approval number: 2023-03/59, date: 24.02.2023). Written informed consent was obtained from all participants prior to enrollment. This study was conducted in accordance with the principles of the "Declaration of Helsinki-Ethical Principles for Medical Research Involving Human Participants".

Statistical Analysis

Cytokine and homocysteine distributions were assessed using the Shapiro-Wilk test and visual inspection (histograms and Q-Q plots). Given the typical right skewness of the data, we also analyzed log10-transformed values. Continuous variables are presented as mean±SD and, where appropriate, median interquartile range (IQR). Between-group comparisons used independent-samples t-tests when assumptions were met, and Mann-Whitney U tests otherwise. Potential outliers were screened visually using box plots and the IQR rule;

sensitivity checks did not change the inference. Alongside p-values, we interpret the direction of effects using two-sided 95% confidence intervals where applicable. Given multiple biomarkers, analyses were treated as exploratory; we did not claim statistical significance after adjustment for multiplicity, and we interpreted findings in light of the increased risk of false positives.

Results

A total of 60 women with POR undergoing IVF were included in the study (Figure 1). The mean age of patients in the POR with sonographic endometrioma group was slightly higher than the POR without sonographic endometrioma group (37.9±5.8 vs. 35.1±5.1 years, respectively; p=0.06), although the difference was not statistically significant. Partner age, body mass index, and baseline ovarian reserve markers, including FSH, AMH, and AFC were comparable between groups. A higher number of previous IVF cycles was observed among patients with POR with sonographic endometrioma, but again, this did not reach statistical significance (3.0±3.1 vs. 1.8±2.1; p=0.07). Demographic and baseline characteristics are summarized in Table 1.

Regarding the FF analysis, homocysteine levels were lower in the POR with sonographic endometrioma group compared to the POR without sonographic endometrioma (1.65±1.53 vs. 4.85±4.14 µmol/L), although the difference was not statistically significant (p=0.277).

Among the 13 inflammatory markers analyzed, most cytokine levels—including IL-1β, IFN-α2, IFN-γ, MCP-1, IL-6, IL-8, IL-10, IL-12p70, IL-17A, IL-18, and IL-23—were lower in the POR group with sonographic endometrioma than in women without sonographic endometrioma. None of these differences reached statistical significance (p>0.05 for all comparisons). Interestingly, two markers—TNF-α and IL-33—were slightly elevated in the POR with sonographic endometrioma group, though again without statistical significance (TNF-α: 10.19±17.38 vs. 6.54±8.72 pg/mL, p=0.761; IL-33: 47.43±72.82 vs. 40.97±61.62 pg/mL, p=0.912).

Across all 14 biomarkers, no between-group differences reached conventional statistical significance. Given right-skewed distributions and multiple comparisons, we conducted complementary nonparametric tests and log10-transformed analyses; both approaches yielded the same inference. Accordingly, we interpret all observed patterns as directional and exploratory rather than definitive. Consistent with this approach, we did not claim statistical significance for any isolated trend after accounting for multiplicity. Despite numerically large mean differences for some biomarkers (e.g., MCP-1, IL-6, IL-18, and homocysteine), wide variances, skewed distributions, and the modest sample size likely reduced statistical power, yielding non-significant p-values; non-parametric and log-transformed analyses led to the same inference.

A full comparison of cytokine and homocysteine levels in FF between the two groups is presented in Table 2.

Although none of the measured biomarkers showed statistically significant differences, the trend toward elevated TNF-α and IL-33 in the POR with sonographic endometrioma group may be consistent with localized inflammatory signaling; however, given the non-significant and imprecise estimates, these observations are exploratory. Conversely, the lower levels of most other cytokines, including IL-6 and MCP-1, suggest a potentially suppressed or dysregulated immune response in the follicular environment of these patients.

Discussion

This exploratory study compared FF cytokines and homocysteine between women with POR who had sonographic endometrioma and those who did not. Across 14 biomarkers, no between-group differences reached conventional statistical significance. Observed patterns were directional: TNF-α and IL-33 tended to be higher, while several cytokines tended to be lower in the endometrioma group, and should be regarded as hypothesis-generating.

TNF-α and IL-33 trends align with proposed inflammatory mechanisms in endometriosis; however, the estimates

Table 1. Socio-demographic parameters (values are mean ± SD unless otherwise specified)

	POR without sonographic endometrioma (n=30)	POR with sonographic endometrioma (n=30)	p-value
Age (years)	35.1±5.1	37.9±5.8	0.06
Partner's age (years)	38.3±6.3	38.1±6.4	0.90
BMI (kg/m^2)	24.7±5.3	25.6±4.8	0.50
FSH (mIU/mL)	17.7±14.3	14.6±9.9	0.32
AMH (ng/mL)	0.47±0.27	0.35±0.31	0.12
AFC (n)	3.1±1.5	2.9±1.5	0.66
Number of previous IVF trials (n)	1.8±2.1	3.0±3.1	0.07

Values are mean ± SD. Units: years (age, partner's age); kg/m^2 (BMI); mIU/mL (FSH); ng/mL (AMH); count (AFC, previous IVF trials) POR: Poor ovarian response, BMI: Body mass index, FSH: Follicle-stimulating hormone, AMH: Anti-Müllerian hormone, AFC: Antral follicle count, SD: Standard deviation, IVF: In vitro fertilization

Table 2. Comparison of follicular fluid cytokine and homocysteine levels between groups

	POR without sonographic endometrioma (n=30) mean ± SD	POR with sonographic endometrioma (n=30) mean ± SD	p-value
Homocysteine (µmol/L)	4.85±4.14	1.65±1.53	0.277
IL-1β (pg/mL)	34.70±32.12	4.37±4.59	0.181
IFN-α2 (pg/mL)	1.44±1.16	0.72±1.25	0.509
IFN-γ (pg/mL)	34.58±31.64	11.10±12.45	0.298
TNF-α (pg/mL)	6.54±8.72	10.19±17.38	0.761
MCP-1 (pg/mL)	895.87±625.14	195.37±193.56	0.137
IL-6 (pg/mL)	26.86±7.54	8.57±13.91	0.116
IL-8 (pg/mL)	1477.26±694.62	756.85±1310.90	0.448
IL-10 (pg/mL)	4.67±3.79	1.34±2.32	0.264
IL-12p70 (pg/mL)	1.10±0.69	0.23±0.03	0.095
IL-17A (pg/mL)	3.41±5.36	0.21±0.22	0.360
IL-18 (pg/mL)	326.61±141.34	94.07±124.85	0.100
IL-23 (pg/mL)	1.80±2.11	0.58±0.00	0.374
IL-33 (pg/mL)	40.97±61.62	47.43±72.82	0.912

Cytokine symbols are standardized as IL-1β, IFN-γ, TNF-α, and MCP-1. Values are mean ± SD. Units are pg/mL for cytokines and µmol/L for homocysteine. Analyses were treated as exploratory given multiple biomarker comparisons. Complementary non-parametric tests and log10-transformed analyses yielded consistent inferences
POR: Poor ovarian response, IL: Interleukin, IFN: Interferon, TNF: Tumor necrosis factor, MCP: Monocyte chemoattractant protein, SD: Standard deviation

were imprecise and not statistically significant; therefore, they should not be interpreted as evidence of a distinct inflammatory profile in POR with endometrioma.

TNF-α has been widely implicated in the inflammatory cascade associated with endometriosis and has been shown to impair oocyte maturation and granulosa cell function[8,12,19]. IL-33, a member of the IL-1 cytokine family, has gained increasing attention for its role in tissue remodeling and immune activation in chronic inflammatory diseases[20]. Our findings align with these observations and suggest a potentially heightened inflammatory state within the follicles of women with both POR and endometriomas.

In contrast, levels of IL-6, IL-1β, IL-8, and MCP-1 were generally lower in the endometrioma group. This counterintuitive finding may indicate an immunological adaptation or exhaustion resulting from chronic local inflammation[20]. Previous studies have suggested that the follicular immune microenvironment in endometriosis may vary depending on disease stage, ovarian reserve, or previous treatment history, all of which could influence cytokine expression profiles[11,18].

Importantly, none of the between-group differences reached conventional statistical significance, and the estimates were imprecise, with wide confidence intervals. Given skewed distributions and multiple biomarker comparisons, these analyses are best considered exploratory. Accordingly, we refrain from inferring a distinct inflammatory profile and instead interpret the observed patterns as directional signals requiring confirmation in larger, outcome-linked cohorts. We note that several large numerical differences were accompanied by wide standard deviations and skewness, which, together with the modest sample size, limit statistical power. The concordance of non-parametric and log-transformed analyses supports the inference that these are directional, non-significant trends.

FF homocysteine levels were also lower in the POR with endometrioma group. Elevated homocysteine is typically considered a negative factor in IVF due to its association with oxidative stress, mitochondrial dysfunction, and impaired methylation capacity[21,22]. The reduced levels in our cohort may reflect an altered metabolic phenotype associated with endometriosis or differences in folate metabolism, although the clinical significance remains unclear.

In addition to cytokine imbalance, alterations in FF composition—including amino acids, lipids, and oxidative stress markers—have been shown to significantly affect oocyte competence and embryo development[14]. Recent approaches using metabolomics support the notion that FF is a dynamic, integrative reflection of both local ovarian physiology and systemic health, making it a promising focus for personalized IVF strategies[14].

Our results are partially consistent with those of Yland et al.[22], who reported differential cytokine patterns in the FF of endometriosis patients, including increased IL-15 and IL-13 and decreased IFN-γ and TNF-α. However, discrepancies may be due to population differences, as their study included

women with normal ovarian reserve, whereas our cohort consisted exclusively of POR patients. The coexistence of endometrioma and poor ovarian reserve likely contributes to a unique immunometabolic profile that warrants further investigation.

Importantly, none of the between-group differences reached conventional statistical significance, and estimates were imprecise with wide confidence intervals. Given skewed distributions and multiple biomarker comparisons, these analyses are best considered exploratory. Accordingly, we refrain from inferring a distinct inflammatory profile and instead interpret the observed patterns as directional signals that require confirmation in larger, outcome-linked cohorts. We note that several numerically large differences were accompanied by wide standard deviations and skewness, which, together with the modest sample size, limit statistical power. The concordance of non-parametric and log-transformed analyses supports the inference that these are directional but non-significant trends.

We did not collect embryological or clinical IVF outcomes (e.g., MII rate, fertilization, blastulation, clinical pregnancy), which precludes correlating FF markers with treatment success in this cohort.

Potential confounders merit consideration. The endometrioma group was slightly older and had undergone more prior IVF cycles, which may influence ovarian response and FF composition. Protocol-related factors (e.g., total gonadotropin dose, trigger-day E2, and follicle counts) can also modulate biomarker levels. In this exploratory dataset, robust multivariable adjustment was not feasible; therefore, we interpret directional patterns with these potential confounders in mind and recommend adjusted analyses in larger cohorts.

Taken together, these non-significant directional findings warrant confirmation in larger, well-phenotyped cohorts that incorporate standardized IVF and pregnancy outcomes and, where possible, detailed endometriosis staging.

Study Limitations

This study has several limitations. First, group allocation relied on the presence of a sonographic endometrioma; the absence of endometrioma does not exclude endometriosis, and occult disease may be present in controls. We did not systematically stage endometriosis or quantify lesion burden beyond the presence of a sonographic endometrioma. Data on endometrioma size, laterality, and prior surgical or medical therapy were incomplete, which may have introduced heterogeneity.

Second, the sample size is modest, increasing imprecision and the risk of type II error, particularly across multiple biomarker comparisons. The modest sample size, in the absence of an a priori power calculation, likely limited our ability to detect small-to-moderate effects.

Third, cytokine distributions are typically skewed. Although we used complementary non-parametric and log-transformed analyses, residual distributional issues cannot be fully excluded.

Moreover, multiple biomarker comparisons increase the risk of false-positive findings; therefore, we treated the analyses as exploratory and claims of statistical significance without adjustment for multiplicity. Residual pre-analytical variability (e.g., subtle blood contamination, storage time, and freeze-thaw effects)cannot be fully excluded despite standardized handling. In addition, residual confounding by age, prior IVF exposure, and stimulation variables cannot be excluded.

Fourth, IVF outcome parameters (e.g., MII rate, fertilization, blastulation, clinical pregnancy) were not collected or reported, which limits clinical interpretability of the findings. Future larger studies should integrate standardized assay performance metrics, correlate FF markers with oocyte, embryo, and pregnancy outcomes, and, where possible, include surgical staging or lesion burden to refine phenotype definitions.

Despite these limitations, our findings underscore the importance of considering both immunological and metabolic markers when evaluating the follicular microenvironment in complex infertility cases. Future research with larger cohorts and functional assays may help elucidate the mechanisms linking endometriosis, ovarian reserve, and follicular health.

Conclusion

In this exploratory study of women with POR undergoing IVF, FF cytokine and homocysteine levels did not differ significantly between patients with and without sonographic endometrioma. Non-significant trends toward higher TNF-α and IL-33 levels and lower homocysteine levels in the endometrioma group should be considered hypothesis-generating. Confirmation in larger, well-phenotyped cohorts incorporating standardized embryological and pregnancy outcomes is needed before clinical inferences can be drawn.

Ethics

Ethics Committee Approval: The study protocol was approved by the Institutional Review Board and the Ethics Committee of Acıbadem University (approval number: 2023-03/59, date: 24.02.2023).

Informed Consent: Written informed consent was obtained from all participants prior to enrollment.

Footnotes

Authorship Contributions

Surgical and Medical Practices: B.E.Ş., Concept: B.E.Ş., E.T., N.Y.S., Y.Ç., B.T., Design: B.E.Ş., Y.Ç., B.T., Data Collection or Processing: B.E.Ş., E.T., İ.Ö.A., N.Y.S., Analysis or Interpretation: B.E.Ş., E.T., N.Y.S., Y.Ç., Literature Search: B.E.Ş., İ.Ö.A., Y.Ç., Writing: B.E.Ş., İ.Ö.A., Y.Ç., B.T.

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