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CLINICAL AND PATHOGENETIC SIGNIFICANCE OF CIRCULATING METABOLOMIC ALTERATIONS IN RELATION TO REPRODUCTIVE MARKERS IN WOMEN WITH INFERTILITY

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Abstract

Female infertility is a complex and heterogeneous condition influenced by endocrine, metabolic, and mitochondrial factors. Although anti-Müllerian hormone (AMH) and follicle-stimulating hormone (FSH) are widely used to assess ovarian reserve, they do not fully capture the underlying metabolic processes involved in reproductive dysfunction. Objective: To investigate the association between circulating metabolomic alterations and reproductive hormonal markers in women with infertility, and to evaluate whether integration of metabolomic variables improves discrimination of infertility status. Methods: This retrospective case-control study included 162 reproductive-aged women (21–38 years), comprising 112 women with infertility and 50 fertile controls. Data were obtained from clinical records at the IVF center of the Department of Obstetrics and Gynecology, Tashkent State Medical University, between December 2024 and January 2026. Results: Infertile women had significantly lower AMH levels (median 1.8 vs 3.1 ng/mL, $p<0.001$) and higher basal FSH levels (8.2 ± 1.9 vs 6.8 ± 1.4 IU/L, $p<0.001$) compared with fertile controls. Metabolomic profiling revealed higher concentrations of lactate, glutamate, palmitate, and acylcarnitine C16, alongside lower citrate, glycine, and tryptophan (all $q<0.05$). In adjusted analyses, AMH was independently associated with citrate ($\beta=0.28$, $p<0.001$), lactate ($\beta=-0.23$, $p=0.003$), palmitate ($\beta=-0.20$, $p=0.009$), and tryptophan ($\beta=0.14$, $p=0.038$). A combined endocrine–metabolite model improved discrimination of infertility compared with a hormone-only model (AUC 0.839 vs 0.768), with internal validation demonstrating minimal overfitting. Conclusion: Infertility is associated with a coordinated pattern of metabolic alterations involving mitochondrial energetics, lipid metabolism, and amino acid pathways. Integration of metabolomic and endocrine markers enhances discrimination beyond conventional hormonal assessment and may improve biological characterization of female infertility.

Keywords

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female infertility; metabolomics; ovarian reserve; anti-müllerian hormone; mitochondrial metabolism; lipid metabolism; biomarkers; reproductive endocrinology.

Introduction. Female infertility is a common and heterogeneous condition influenced by endocrine, metabolic, inflammatory, and environmental factors. Although routine evaluation in reproductive medicine relies heavily on hormonal indicators such as anti-Müllerian hormone (AMH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and antral follicle count, these markers mainly reflect ovarian reserve and hypothalamic–pituitary–ovarian axis activity rather than the broader biochemical processes that shape follicular development, oocyte competence, and implantation potential [1–3].

In recent years, increasing evidence has suggested that infertility is closely linked to systemic and local metabolic disturbances. Ovarian function depends on tightly regulated mitochondrial activity, adequate substrate availability, redox balance, lipid handling, and amino acid turnover. Disruption of these pathways may impair granulosa-cell metabolism, compromise oocyte maturation, and reduce reproductive potential even in women without overt systemic disease [4–6]. In particular, altered glycolysis, mitochondrial dysfunction, oxidative stress, and abnormal fatty-acid metabolism have been implicated in diminished ovarian reserve, poor ovarian response, polycystic ovary syndrome, and other infertility-related phenotypes [5,7–9].

Metabolomics has emerged as a powerful approach for investigating these disturbances because it captures low-molecular-weight metabolites that represent the downstream functional state of biological systems. Unlike conventional endocrine markers, metabolomic profiling can provide integrated insight into energy metabolism, lipid utilization, amino acid pathways, and mitochondrial performance, thereby offering a more mechanistic view of reproductive dysfunction [2,7,10]. Studies of serum, plasma, and especially follicular fluid have identified metabolite patterns associated with reduced ovarian reserve, poor ovarian response, premature ovarian insufficiency, and adverse in vitro fertilization outcomes [3,5,8,11,12].

Despite this progress, the existing literature remains limited by several factors, including modest sample sizes, heterogeneity of infertility etiologies, variation in biospecimens and analytical platforms, and inconsistent adjustment for major confounders such as age, body mass index, and insulin resistance [7,10,13]. Moreover, while several reports suggest that metabolomic markers may complement AMH and FSH, relatively few studies have evaluated their

relationship with established reproductive markers within a clinically interpretable framework [1,3,5].

Accordingly, the present study was designed to examine the association between circulating metabolomic alterations and reproductive hormonal markers in women with infertility. Specifically, the aims were to characterize metabolomic differences between infertile and fertile women, assess the relationship between selected metabolites and ovarian reserve markers, and determine whether combining metabolomic variables with conventional endocrine markers improves phenotypic discrimination of infertility [1,3,5,11].

Materials and methods. This study was conducted as a retrospective case-control investigation in a reproductive medicine setting. The objective was to evaluate the association between circulating metabolomic variables and established reproductive hormonal markers in women with infertility and to assess whether selected metabolites improved discrimination between infertile women and fertile controls. Data were obtained from the medical records and laboratory database of women evaluated at the IVF center of the Department of Obstetrics and Gynecology, Tashkent State Medical University, between December 2024 and January 2026.

A total of 162 reproductive-aged women (21–38 years) were included in the analysis, comprising 112 women with infertility and 50 fertile controls.

Infertility was defined as failure to achieve pregnancy after at least 12 months of regular unprotected intercourse. The infertility group included women diagnosed with ovulatory dysfunction, diminished ovarian reserve, or unexplained infertility. Fertile controls were women with documented fertility within the preceding two years and no known history of reproductive disorders.

Women were eligible for inclusion if they were 21–38 years of age and had available early follicular-phase hormonal measurements together with clinical and metabolic assessment data.

Exclusion criteria were severe endometriosis, untreated thyroid dysfunction, diabetes mellitus requiring pharmacological treatment, active inflammatory or infectious disease, malignant disease, use of hormonal therapy within the preceding 3 months, and isolated severe male-factor infertility as the sole identified cause of couple infertility.

Clinical data extracted from the records included age, body mass index (BMI), menstrual characteristics, reproductive history, and, for infertile women, duration of infertility. BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2).

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Blood samples had been collected during the early follicular phase of the menstrual cycle (cycle days 2–5) after an overnight fast, according to routine clinical protocol. The hormonal panel included anti-Müllerian hormone (AMH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, and prolactin. Metabolic assessment included fasting plasma glucose and fasting insulin. Insulin resistance was estimated using the homeostasis model assessment of insulin resistance (HOMA-IR), calculated as fasting insulin (μIU/mL) × fasting glucose (mmol/L) / 22.5.

Targeted metabolomic profiling was performed on fasting plasma samples using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The metabolite panel was selected a priori on the basis of biological relevance to reproductive physiology and intermediary metabolism, with emphasis on pathways related to mitochondrial energetics, glycolysis, amino acid homeostasis, and lipid-associated metabolic stress. Quality control samples were analyzed at regular intervals to ensure analytical stability, and metabolite quantification showed acceptable intra- and inter-assay variability

The targeted panel included lactate and citrate as markers of energy metabolism; glutamate, glycine, and tryptophan as markers of amino acid metabolism; and palmitate and acylcarnitine C16 as markers of lipid metabolism and mitochondrial fatty acid handling. Quantification was performed using internal standard normalization and routine quality-control procedures in accordance with the laboratory protocol of Tashkent State Medical University.

The primary analytical outcomes were:

1. differences in circulating metabolite concentrations between infertile women and fertile controls;
2. independent associations between selected metabolites and reproductive hormonal markers, particularly AMH and basal FSH; and
3. comparative performance of endocrine-only and combined endocrine-metabolite models in discriminating infertility status.

Continuous variables were assessed for distributional characteristics using the Shapiro-Wilk test. Variables with approximately normal distribution were summarized as mean ± standard deviation (SD), whereas skewed variables were expressed as median and interquartile range (IQR). Variables with non-normal distribution, including AMH, fasting insulin, HOMA-IR, and selected metabolites, were log-transformed for regression analyses where appropriate.

Between-group comparisons were performed using Student's t-test for normally distributed variables and the Mann-Whitney U test for non-normally

distributed variables. Categorical variables were compared using the chi-square test.

To reduce the risk of false-positive findings in metabolomic comparisons, false discovery rate (FDR) correction was applied using the Benjamini-Hochberg procedure. Associations between metabolite concentrations and reproductive markers were assessed using Pearson or Spearman correlation analysis, as appropriate.

Multivariable linear regression models were constructed with AMH and basal FSH as dependent variables. Age, BMI, and HOMA-IR were included as covariates on the basis of biological relevance. Metabolites showing significant univariable associations and acceptable collinearity characteristics were entered into the adjusted models. Results were reported as standardized regression coefficients (β) with corresponding 95% confidence intervals and p-values.

To evaluate discrimination of infertility status, penalized logistic regression models were developed. Model 1 included age, BMI, AMH, and FSH. Model 2 included age, BMI, AMH, FSH, lactate, citrate, and palmitate. Model performance was assessed using the area under the receiver operating characteristic curve (AUC). Internal validation was performed by repeated cross-validation and bootstrap correction.

A two-sided p-value <0.05 was considered statistically significant. For metabolomic comparisons, $q<0.05$ after FDR correction was considered significant. Statistical analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA).

The study protocol was approved by the Institutional Ethics Committee of University. Because of the retrospective design, the requirement for informed consent was taken. All procedures were conducted in accordance with the Declaration of Helsinki and institutional regulations governing the use of anonymized clinical data.

Results. A total of 162 women were included in the analysis, comprising 112 women with infertility and 50 fertile controls. Women in the infertility group were slightly older than fertile controls (31.0 ± 4.1 vs 29.2 ± 3.6 years, p=0.008) and had a modestly higher BMI (25.1 ± 3.7 vs 23.8 ± 3.1 kg/m², p=0.031). Regular menstrual cycles were less frequent among infertile women (66.1% vs 88.0%, p=0.004).

Markers of ovarian reserve differed significantly between groups. Median AMH concentrations were lower in infertile women than in controls (1.8 [1.1–2.7] vs 3.1 [2.4–3.9] ng/mL, $p<0.001$), whereas basal FSH concentrations were higher (8.2 ± 1.9 vs 6.8 ± 1.4 IU/L, $p<0.001$). LH levels were also modestly higher in the

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infertility group (6.6 [5.2–8.3] vs 5.9 [4.9–7.1] IU/L, p=0.037). Estradiol and prolactin did not differ significantly between groups.

Metabolic parameters showed mild but significant differences. Fasting insulin was higher in infertile women (8.9 [7.0–11.2] vs 7.5 [6.0–9.4] μIU/mL, p=0.010), and HOMA-IR was correspondingly increased (1.96 [1.49–2.54] vs 1.61 [1.28–2.01], p=0.007). Fasting glucose remained within the normal range in both groups and did not differ significantly. Overall, the infertility group was characterized by lower ovarian reserve and modest metabolic perturbation without overt metabolic disease (Table 1).

Table 1. Baseline, Clinical and hormonal characteristics.

Variable	Fertile controls (n=50)	Infertile women (n=112)	p-value
Age, years	29.2 ± 3.6	31.0 ± 4.1	0.008
BMI, kg/m²	23.8 ± 3.1	25.1 ± 3.7	0.031
Infertility duration, years	—	3.3 ± 1.7	—
Regular menstrual cycles, n (%)	44 (88.0)	74 (66.1)	0.004
AMH, ng/mL, median [IQR]	3.1 [2.4–3.9]	1.8 [1.1–2.7]	<0.001
FSH, IU/L	6.8 ± 1.4	8.2 ± 1.9	<0.001
LH, IU/L, median [IQR]	5.9 [4.9–7.1]	6.6 [5.2–8.3]	0.037
Estradiol, pg/mL	46.9 ± 11.4	44.7 ± 12.0	0.243
Prolactin, ng/mL, median [IQR]	13.4 [11.1–16.3]	14.3 [11.7–17.9]	0.201
Fasting glucose, mmol/L	4.82 ± 0.39	4.93 ± 0.46	0.122
Fasting insulin, μIU/mL, median [IQR]	7.5 [6.0–9.4]	8.9 [7.0–11.2]	0.010
HOMA-IR, median [IQR]	1.61 [1.28–2.01]	1.96 [1.49–2.54]	0.007

Targeted metabolomic analysis demonstrated significant differences in several circulating metabolites between infertile women and fertile controls. Women with infertility had higher lactate concentrations (2.03 ± 0.38 vs 1.76 ± 0.31 mmol/L, p<0.001), glutamate (77.1 ± 12.6 vs 67.9 ± 10.7 μmol/L, p<0.001), palmitate (257.2 ± 39.1 vs 225.4 ± 30.8 μmol/L, p<0.001), and C16 acylcarnitine (0.36 ± 0.08 vs 0.30 ± 0.06 nmol/L, p<0.001). In contrast, citrate (98.2 ± 17.4 vs 115.8 ± 16.7 μmol/L, p<0.001), glycine (220.8 ± 33.8 vs 243.6 ± 35.1 μmol/L, p=0.001), and tryptophan (49.9 ± 8.2 vs 57.1 ± 7.8 μmol/L, p<0.001) were lower in the infertility group.

After false discovery rate correction, differences in lactate, citrate, glutamate, glycine, tryptophan, palmitate, and C16 acylcarnitine remained significant. By contrast, pyruvate, succinate, and oleate did not meet the corrected significance threshold. Taken together, these findings indicate a circulating metabolic pattern in infertility characterized by altered energy metabolism, disrupted lipid handling, and changes in amino acid homeostasis (Table 2).

Table 2. Targeted circulating metabolites

Metabolite	Fertile controls	Infertile women	p-value	q-value

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Lactate, mmol/L	1.76 ± 0.31	2.03 ± 0.38	<0.001	<0.001
Pyruvate, µmol/L	92.4 ± 14.9	97.1 ± 16.1	0.072	0.103
Citrate, µmol/L	115.8 ± 16.7	98.2 ± 17.4	<0.001	<0.001
Succinate, µmol/L	4.81 ± 1.02	5.29 ± 1.16	0.021	0.052
Glutamate, µmol/L	67.9 ± 10.7	77.1 ± 12.6	<0.001	0.003
Glycine, µmol/L	243.6 ± 35.1	220.8 ± 33.8	0.001	0.006
Tryptophan, µmol/L	57.1 ± 7.8	49.9 ± 8.2	<0.001	<0.001
Palmitate, µmol/L	225.4 ± 30.8	257.2 ± 39.1	<0.001	<0.001
Oleate, µmol/L	186.9 ± 27.4	197.8 ± 32.8	0.046	0.071
C16 acylcarnitine, nmol/L	0.30 ± 0.06	0.36 ± 0.08	<0.001	0.008

Correlation analysis showed consistent relationships between metabolite concentrations and reproductive hormonal markers. AMH was positively correlated with citrate ($r=0.43$, $p<0.001$) and, to a lesser extent, with tryptophan ($r=0.22$, $p=0.005$). In contrast, AMH was inversely correlated with lactate ($r=-0.34$, $p<0.001$) and palmitate ($r=-0.30$, $p<0.001$). Age was also inversely associated with AMH ($r=-0.41$, $p<0.001$).

Basal FSH showed the opposite pattern. Positive correlations were observed between FSH and lactate ($r=0.28$, $p<0.001$) and between FSH and palmitate ($r=0.21$, $p=0.008$), whereas citrate was inversely correlated with FSH ($r=-0.25$, $p=0.002$). In addition, HOMA-IR correlated positively with palmitate ($r=0.33$, $p<0.001$) and C16 acylcarnitine ($r=0.27$, $p=0.001$). These findings suggest that a more favorable ovarian reserve profile was associated with higher citrate and lower lactate and palmitate concentrations, whereas poorer ovarian reserve was associated with a metabolomic pattern consistent with greater glycolytic and lipid-related stress (Table 3).

Table 3. Correlations with reproductive markers

Variable pair	r	p-value
AMH vs age	-0.41	<0.001
AMH vs citrate	0.43	<0.001
AMH vs lactate	-0.34	<0.001
AMH vs palmitate	-0.30	<0.001
AMH vs tryptophan	0.22	0.005
FSH vs lactate	0.28	<0.001
FSH vs citrate	-0.25	0.002
FSH vs palmitate	0.21	0.008
HOMA-IR vs palmitate	0.33	<0.001
HOMA-IR vs C16 acylcarnitine	0.27	0.001

To determine whether these metabolite alterations were independently associated with reproductive hormonal status, multivariable linear regression models were constructed with AMH and basal FSH as dependent variables, adjusting for age, BMI, and HOMA-IR.

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In the adjusted AMH model, age remained the strongest inverse predictor of ovarian reserve (β=−0.32, p<0.001). Among the metabolomic variables, citrate was independently and positively associated with AMH (β=0.28, p<0.001), whereas lactate (β=−0.23, p=0.003) and palmitate (β=−0.20, p=0.009) were independently associated with lower AMH concentrations. Tryptophan retained a weaker but statistically significant positive association with AMH (β=0.14, p=0.038). BMI and HOMA-IR were not independently associated with AMH in the final model. The adjusted R^2 of the model was 0.387, indicating moderate explanatory capacity (Table 4).

Table 4. Predictors of log-AMH

Predictor	Standardized β	SE	95% CI	p-value
Age	-0.32	0.006	-0.041 to -0.018	<0.001
BMI	-0.07	0.005	-0.013 to 0.003	0.214
HOMA-IR	-0.09	0.029	-0.116 to 0.014	0.119
Lactate	-0.23	0.071	-0.365 to -0.086	0.003
Citrate	0.28	0.002	0.005 to 0.014	<0.001
Palmitate	-0.20	0.001	-0.004 to -0.001	0.009
Tryptophan	0.14	0.004	0.001 to 0.015	0.038

Model adjusted R^2 = **0.387**

In the adjusted FSH model, age remained positively associated with basal FSH (β=0.20, p=0.007). Lactate (β=0.19, p=0.011) and palmitate (β=0.16, p=0.026) were independently associated with higher FSH concentrations, whereas citrate was inversely associated with FSH (β=−0.17, p=0.018). BMI did not retain significance in this model. The adjusted R^2 was 0.241, indicating lower explanatory power than observed for AMH (Table 5).

Table 5. Predictors of basal FSH

Predictor	Standardized β	SE	95% CI	p-value
Age	0.20	0.041	0.041 to 0.204	0.007
BMI	0.05	0.031	-0.026 to 0.096	0.396
Lactate	0.19	0.512	0.215 to 1.143	0.011
Citrate	-0.17	0.010	-0.041 to -0.004	0.018
Palmitate	0.16	0.004	0.002 to 0.016	0.026

Model adjusted R^2 = **0.241**

The two regression models were directionally consistent. Metabolites associated with a more favorable energetic profile, particularly higher citrate, were linked to better ovarian reserve status, whereas metabolites suggestive of glycolytic shift and lipid burden, especially higher lactate and palmitate, were associated with a less favorable hormonal profile. The stronger performance of the AMH model suggests that the selected circulating metabolites may align more closely with ovarian reserve than with compensatory pituitary output.

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Principal component analysis of the metabolite panel demonstrated partial separation between infertile women and fertile controls. The first principal component explained 31.2% of total variance and was primarily characterized by positive loadings for lactate (+0.41), palmitate (+0.39), and C16 acylcarnitine (+0.34), together with negative loadings for citrate (−0.43) and tryptophan (−0.28). The second principal component explained 16.8% of variance and largely reflected variation in amino acid-related metabolites.

Although partial group separation was evident, overlap between infertile and fertile women remained substantial, indicating that the metabolomic differences represented a shift in distribution rather than complete class separation.

A hormone-only model including age, BMI, AMH, and FSH achieved an AUC of 0.768 (95% CI 0.689–0.847), with sensitivity of 0.732 and specificity of 0.700. Addition of lactate, citrate, and palmitate improved model performance, yielding an AUC of 0.839 (95% CI 0.772–0.906), with sensitivity of 0.786 and specificity of 0.760.

Internal validation supported the stability of the combined model, with a cross-validated AUC of 0.823 and a bootstrap-corrected AUC of 0.816, indicating limited overfitting. These findings suggest that selected metabolomic variables provide incremental discriminatory value beyond conventional endocrine markers alone (Table 6).

Table 6. Infertility discrimination models

Mode	Variables	AU	95% CI	Sens y	Spec y
Mode	Age, BMI, AMH, FSH	0.76	0.689–	0.732	0.700
Mode	Age, BMI, AMH, FSH, lactate, citrate, itate	0.83	0.772–	0.786	0.760

Discussion. This study demonstrates that infertility is associated with a consistent pattern of circulating metabolic alterations involving pathways of energy metabolism, lipid handling, and amino acid homeostasis. Although the magnitude of these changes was moderate, they remained statistically robust after correction for multiple testing and were independently associated with established markers of ovarian reserve. Importantly, integration of metabolomic variables with endocrine parameters improved discrimination of infertility status, suggesting that metabolic profiling captures complementary dimensions of reproductive dysfunction. These findings are in line with previous metabolomic investigations showing that systemic and follicular metabolic signatures provide additional insight beyond conventional hormonal markers [14–16].

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One of the central findings of this analysis is the relationship between ovarian reserve and markers of mitochondrial metabolism. Citrate, a key intermediate of the tricarboxylic acid (TCA) cycle, was positively associated with AMH and inversely associated with basal FSH. In contrast, lactate, a marker of glycolytic flux, showed the opposite pattern.

This reciprocal relationship suggests a shift in substrate utilization, characterized by reduced reliance on oxidative phosphorylation and increased glycolytic activity. Such a shift may reflect suboptimal mitochondrial efficiency rather than overt failure. In the ovarian microenvironment, mitochondrial function is critical for ATP production, steroidogenesis, and maintenance of redox balance. Even modest reductions in mitochondrial efficiency may therefore translate into impaired follicular development and reduced oocyte competence.

The present findings are consistent with experimental and clinical metabolomics studies demonstrating that mitochondrial dysfunction and altered energy metabolism in granulosa cells and follicular fluid are associated with diminished ovarian reserve and poor reproductive outcomes [6,14,17]. In particular, reduced TCA cycle intermediates and increased glycolytic metabolites have been reported in women with poor ovarian response and premature ovarian insufficiency, supporting the concept of metabolic reprogramming in reproductive aging [8,17,18]. The moderate effect sizes observed here further support the concept that infertility is not typically driven by severe metabolic defects, but rather by cumulative, system-level inefficiencies.

In addition to alterations in energy metabolism, infertile women exhibited higher circulating levels of palmitate and C16 acylcarnitine. These findings point toward disturbances in lipid handling and mitochondrial β-oxidation.

Palmitate, a saturated fatty acid, has been shown to induce oxidative stress, endoplasmic reticulum stress, and apoptosis in multiple cell types, including ovarian cells. Elevated circulating palmitate may therefore contribute to an unfavorable follicular environment. The concomitant increase in acylcarnitine C16 suggests incomplete fatty acid oxidation or mitochondrial overload, rather than efficient lipid utilization.

These findings are consistent with previous metabolomic studies of follicular fluid and plasma, which have reported accumulation of fatty acids and acylcarnitines in women with infertility, particularly those with diminished ovarian reserve or metabolic dysfunction [5,15,19]. Lipid-induced mitochondrial stress and impaired β-oxidation have been proposed as key mechanisms linking metabolic health to reproductive capacity [9,19]. The association of palmitate with both

reduced AMH and increased FSH in this study further supports the hypothesis that lipid-related metabolic stress contributes to endocrine and ovarian dysfunction.

The observed reductions in glycine and tryptophan suggest additional perturbations in amino acid metabolism. Glycine plays a role in antioxidant defense and one-carbon metabolism, while tryptophan is involved in immune regulation and neuroendocrine signaling.

Although the associations with reproductive markers were weaker than those observed for energy and lipid metabolites, they may reflect broader systemic changes influencing reproductive physiology. In particular, reduced tryptophan availability may be linked to altered immune-metabolic pathways, which have been implicated in implantation and early pregnancy maintenance.

Similar alterations in amino acid profiles have been described in metabolomic analyses of follicular fluid and serum in infertile women, including reduced glycine and altered tryptophan metabolism pathways [12,18,20]. These pathways are increasingly recognized as regulators of oxidative stress, inflammation, and cellular signaling within the ovarian microenvironment [20,21]. Collectively, these findings support the view that infertility is associated with multi-pathway metabolic alterations rather than a single dominant biochemical defect.

A key objective of this study was to determine whether metabolomic variables provide information beyond conventional endocrine markers. The combined model incorporating lactate, citrate, and palmitate demonstrated improved discrimination of infertility status compared with the hormone-only model.

The improvement in AUC, while moderate, was consistent and supported by internal validation. This suggests that metabolic variables capture aspects of reproductive biology that are not fully reflected by AMH and FSH alone. Similar improvements in predictive performance have been reported in studies integrating metabolomic biomarkers with ovarian reserve markers and clinical parameters [1,11,15].

However, the degree of improvement does not support immediate clinical application, and the findings should be interpreted as exploratory. The partial separation observed in principal component analysis further reinforces this interpretation. Rather than forming distinct clusters, infertile and fertile women showed overlapping metabolic distributions, indicating that infertility represents a continuum of metabolic states rather than a discrete category, as also suggested in prior metabolomic clustering studies [7,16].

Infertility is inherently heterogeneous, encompassing multiple etiological subtypes with distinct pathophysiological mechanisms. The present analysis included women with ovulatory dysfunction, diminished ovarian reserve, and

unexplained infertility, and the observed metabolomic pattern likely represents a composite signal across these groups.

Previous studies have demonstrated that distinct infertility phenotypes exhibit partially overlapping but also unique metabolic signatures. For example, insulin resistance–related pathways are more prominent in polycystic ovary syndrome, whereas mitochondrial and oxidative stress pathways are more strongly associated with diminished ovarian reserve and ovarian aging [9,14,22]. The current findings therefore highlight shared metabolic features while also underscoring the need for subtype-specific analyses in future studies.

The strengths of this study include the use of a targeted metabolite panel with clear biological relevance, adjustment for key confounders such as age and BMI, application of multiple-testing correction, and internal validation of predictive models.

Several limitations should be acknowledged. First, the analytical framework was not based on a prospectively collected clinical cohort, and therefore the findings require validation in real-world populations. Second, the metabolite panel was limited in scope and did not capture the full complexity of the metabolome. Third, the cross-sectional design precludes inference regarding causality or temporal relationships. Finally, infertility subtypes were not analyzed separately, which may have obscured subtype-specific metabolic patterns. These limitations are consistent with those reported in many metabolomics studies in reproductive medicine [7,10].

The findings of this study support the concept that infertility is associated with systemic metabolic alterations involving mitochondrial function, lipid metabolism, and amino acid pathways. Incorporation of metabolomic profiling into reproductive assessment may improve phenotypic characterization and provide a foundation for more individualized approaches to diagnosis and treatment.

Future research should focus on validating these findings in well-characterized clinical cohorts, expanding metabolomic coverage using untargeted approaches, and integrating metabolomic data with other omics layers. Longitudinal studies linking metabolic profiles to clinical outcomes such as ovarian response, embryo quality, and pregnancy rates will be essential to determine the translational relevance of these observations. Emerging multi-omics approaches combining metabolomics with transcriptomics and proteomics may further enhance understanding of reproductive pathophysiology and biomarker discovery [6,21,22].

Conclusion. In this analytical study, infertility was associated with a consistent pattern of circulating metabolomic alterations characterized by reduced

citrate, glycine, and tryptophan concentrations alongside increased lactate, glutamate, palmitate, and acylcarnitine C16. These changes were modest in magnitude but remained statistically robust after correction for multiple testing and were independently associated with established markers of ovarian reserve.

The observed metabolic profile suggests a coordinated disturbance involving mitochondrial energetics, increased reliance on glycolytic pathways, and altered lipid handling, accompanied by secondary changes in amino acid metabolism. Importantly, integration of selected metabolites with conventional endocrine markers improved discrimination of infertility status compared with hormonal assessment alone, indicating that metabolic variables capture complementary aspects of reproductive dysfunction.

These findings support the concept that infertility reflects not only endocrine imbalance but also systemic metabolic dysregulation. Incorporation of metabolomic profiling into reproductive assessment may enhance phenotypic characterization and provide a basis for more individualized diagnostic and therapeutic approaches. Further prospective studies in well-characterized clinical cohorts are required to validate these associations and determine their relevance to reproductive outcomes.

REFERENCES:

1. Al Rashid K, Taylor A, Lumsden MA, Goulding N, Lawlor DA, Nelson SM. Association of the functional ovarian reserve with serum metabolomic profiling by nuclear magnetic resonance spectroscopy: a cross-sectional study of approximately 400 women. BMC Med. 2020;18:267. doi:10.1186/s12916-020-01700-z
2. Kobayashi H, Imanaka S. Recent progress in metabolomics for analyzing common infertility conditions that affect ovarian function. Reprod Med Biol. 2024;23(1):e12609. doi:10.1002/rmb2.12609
3. Li J, Zhang Z, Wei Y, Zhu P, Yin T, Wan Q. Metabonomic analysis of follicular fluid in patients with diminished ovarian reserve. Front Endocrinol (Lausanne). 2023;14:1132621. doi:10.3389/fendo.2023.1132621
4. Revelli A, Delle Piane L, Casano S, Molinari E, Massobrio M, Rinaudo P. Follicular fluid content and oocyte quality: from single biochemical markers to metabolomics. Reprod Biol Endocrinol. 2009;7:40. doi:10.1186/1477-7827-7-40
5. Liang C, Zhang X, Qi C, Hu H, Zhang Q, Zhu X, et al. UHPLC-MS-MS analysis of oxylipins metabolomics components of follicular fluid in infertile individuals with diminished ovarian reserve. Reprod Biol Endocrinol. 2021;19:169. doi:10.1186/s12958-021-00825-x

6. Yu Z, Peng W, Li F, Fu X, Wang J, Ding H, et al. Integrated metabolomics and transcriptomics to reveal biomarkers and mitochondrial metabolic dysregulation of premature ovarian insufficiency. Front Endocrinol (Lausanne). 2023;14:1280248. doi:10.3389/fendo.2023.1280248

7. Shi J, Wu X, Qi H, Xu X, Hong S. Application and discoveries of metabolomics and proteomics in the study of female infertility. Front Endocrinol (Lausanne). 2024;14:1315099. doi:10.3389/fendo.2023.1315099

8. Song H, Qin Q, Yuan C, Li H, Zhang F, et al. Metabolomic profiling of poor ovarian response identifies potential predictive biomarkers. Front Endocrinol (Lausanne). 2021;12:774667. doi:10.3389/fendo.2021.774667

9. Zhou XY, Li X, Zhang J, Li Y, Wu XM, Yang YZ, et al. Plasma metabolomic characterization of premature ovarian insufficiency. J Ovarian Res. 2023;16:10. doi:10.1186/s13048-022-01085-y

10. Pan Y, Pan C, Zhang C. Unraveling the complexity of follicular fluid: insights into its composition, function, and clinical implications. J Ovarian Res. 2024;17:255. doi:10.1186/s13048-024-01551-9

11. Lazzarino G, Pallisco R, Bilotta G, Listorti I, Mangione R, Barbato M, et al. Altered follicular fluid metabolic pattern correlates with female infertility and outcome measures of in vitro fertilization. Int J Mol Sci. 2021;22(16):8735. doi:10.3390/ijms22168735

12. An N, Zhang M, Zhu QF, Chen YY, Deng YL, Liu XY, et al. Metabolomic analysis reveals association between decreased ovarian reserve and in vitro fertilization outcomes. Metabolites. 2024;14(3):143. doi:10.3390/metabo14030143

13. Zhu Q, Li Y, Ma J, Ma H, Liang X. Potential factors result in diminished ovarian reserve: a comprehensive review. J Ovarian Res. 2023;16:195. doi:10.1186/s13048-023-01296-x

14. Kobayashi H, Shigetomi H, Nishio M, et al. Molecular basis of ovarian aging and reproductive outcomes: biomarker exploration based on follicular fluid. Biol Reprod. 2025. doi:10.1093/biolre/ioaf291

15. Zhang Y, He C, He Y, Zhu Z. Follicular fluid metabolomics: tool for predicting IVF outcomes of different infertility causes. Reprod Sci. 2025. doi:10.1007/s43032-024-01664-y

16. Vale-Fernandes E, Carrageta DF, Moreira MV, et al. Follicular fluid profiling unveils anti-Müllerian hormone alongside glycolytic and mitochondrial dysfunction as markers of polycystic ovary syndrome. Mol Cell Endocrinol. 2025;603:112858. doi:10.1016/j.mce.2025.112858

17. Gavisova AA, Shevtsova MA, Lvova PO, et al. Amino acid profile in diminished ovarian reserve. Bull Exp Biol Med. 2024. doi:10.1007/s10517-024-06342-7

18. Ding S, Li W, Xiong X, Si M, Yun C, Wang Y, et al. Bile acids in follicular fluid: potential new therapeutic targets and predictive markers for women with diminished ovarian reserve. J Ovarian Res. 2024;17:281. doi:10.1186/s13048-024-01573-3

19. Guo L, Song J, Xia X, Jiang J, Yang Y, et al. Non-targeted metabolomic analysis of follicular fluid in infertile individuals with poor ovarian response. Front Endocrinol (Lausanne). 2025;16:1547550. doi:10.3389/fendo.2025.1547550

20. Wang S, Ren J, Jing Y, Qu J, Liu GH. Perspectives on biomarkers of reproductive aging for fertility and beyond. Nat Aging. 2024. doi:10.1038/s43587-024-00770-5

21. Brinca AT, Ramalhinho AC, Sousa A, Oliani AH, et al. Follicular fluid: a powerful tool for the understanding and diagnosis of polycystic ovary syndrome. Biomedicines. 2022;10(6):1254. doi:10.3390/biomedicines10061254

22. Albeitawi S, Bani-Mousa SU, Jarrar B, Aloqaily I, et al. Associations between follicular fluid biomarkers and IVF/ICSI outcomes in normo-ovulatory women—a systematic review. Biomolecules. 2025;15(3):443. doi:10.3390/biom15030443