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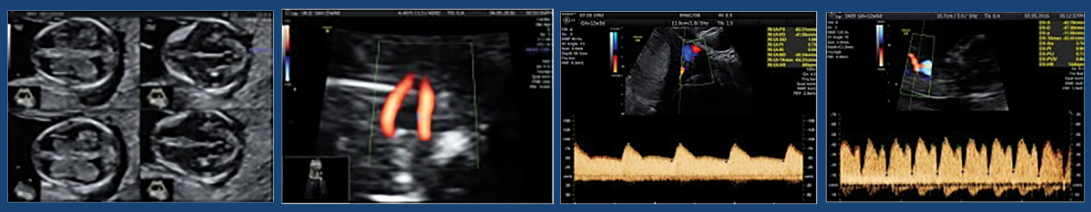

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Cover Picture: Dilek et al. Early diagnosis opportunity in first trimester screening

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Editorial Office

Address: Abdi İpekçi Cad. 2/7 34367 Nişantaşı, İstanbul-Turkey
Phone: +90 212 241 45 45
Fax: +90 212 241 44 08
E-mail: tajev@tajev.org

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Aims and Scope

Journal of the Turkish-German Gynecological Association is the official, open access publication of the Turkish-German Gynecological Education and Research Foundation and Turkish-German Gynecological Association and is published quarterly on March, June, September and December. The publication language of the journal is English. Manuscripts are reviewed in accordance with "double-blind peer review" process for both reviewers and authors.

The target audience of Journal of the Turkish-German Gynecological Association includes gynecologists and primary care physicians interested in gynecology practice. It publishes original works on all aspects of obstertrics and gynecology. The aim of Journal of the Turkish-German Gynecological Association is to publish high quality original research articles. In addition to research articles, reviews, editorials, letters to the editor, diagnostic puzzle are also published. Suggestions for new books are also welcomed. Journal of the Turkish-German Gynecological Association does not charge any fee for article submission or processing.

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The ''Journal of the Turkish-German Gynecological Association'' (EISSN 1309-0380; Abbreviated as "J Turk Ger Gynecol Assoc") is the official, open access publication of the Turkish-German Gynecological Education and Research Foundation and the Turkish-German Gynecological Association. Formerly named "ARTEMIS", the journal is published quarterly (March, June, September, December) in English and publishes original peer-reviewed articles, reviews, and commentaries in the fields of Gynecology, Gynecologic Oncology, Endocrinology & Reproductive Medicine and Obstetrics. Case reports are not accepted for publication. Reviews will be considered for publication only if they are prepared by authors who have at least three published manuscripts in international peer reviewed journals and these studies should be cited in the review. Otherwise only invited reviews will be considered for peer review from qualified experts in the area.

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PRISMA for preferred reporting items for systematic reviews and meta-analyses (Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 2009; 6(7): e1000097.) (http://www.prisma-statement.org/),

STARD checklist for the reporting of studies of diagnostic accuracy (Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, et al, for the STARD Group. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. Ann Intern Med 2003;138:40-4.) (http://www.stard-statement.org/),

STROBE statement-checklist of items that should be included in reports of observational studies (http://www.strobe-statement.org/),

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In experimental animal studies, the authors should indicate that the procedures followed were in accordance with animal rights as per the Guide for the Care and Use of Laboratory Animals (http://oacu.od.nih.gov/regs/guide/guide.pdf) and they should obtain animal ethics committee approval.

The editors reserve the right to reject manuscripts that do not comply with the above-mentioned requirements. The author will be held responsible for false statements or for failure to fulfil the above mentioned requirements.

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A separate title page should be submitted with all submissions and should include the title of the article, name(s), affiliations and major

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All manuscripts should be accompanied by an abstract. A structured abstract is required with original articles and it should include the following subheadings: Objective, Material and Methods, Results and Conclusion. A structured abstract is not required with review articles. The abstract should be limited to 250 words for original articles and review articles.

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Below the abstract provide 3 to 5 Keywords. Abbreviations should not be used as Keywords. Keywords should be picked from the Medical Subject Headings (MeSH) list (www.nlm.nih.gov/mesh/MBrowser.html).

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State concisely the purpose and rationale for the study and cite only the most pertinent references as background.

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Describe the plan, the patients, experimental animals, material and controls, the methods and procedures utilized, and the statistical method(s) employed. In addition to the normal peer review procedure, all randomized controlled trials (RCTs) submitted to the journal are sent to members of a team of professional medical statisticians for reviewing.

Address "Institutional Review Board" issues as stated above. State the generic names of the drugs with the name and country of the manufactures. Provide information on informed consent and ethics committee approval.

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Present the detailed findings supported with statistical methods. Figures and tables should supplement, not duplicate the text; presentation of data in either one or the other will suffice. Emphasize only your important observations; do not compare your observations with those of others. Such comparisons and comments are reserved for the discussion section.

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Book;

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Contents

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Editorial

Dear Colleagues,

It is my great pleasure to introduce the first issue of the "Journal of the Turkish-German Gynecological Association (J Turk Ger Gynecol Assoc)" in the publishing year of 2026. This issue is consisted of six articles, and one meta-analysis that we hope you will read with interest. Also you may have the oppurtunity to read the quiz. Here we share some of our favorite articles that were published in this issue of the journal.

Ovarian cancer continues to be a major global health concern due to its high mortality rate and late-stage detection. Their different molecular profiles, which in turn determine their varying clinical behaviors, are the fundamental differences between high-grade (HGOC) and low-grade ovarian cancer (LGOC). Finding prospective biomarkers and treatment targets may benefit from a deeper comprehension of the molecular mechanisms underlying the progression of ovarian cancer. A study employing bioinformatics analysis to identify key genes and elucidate their potential molecular mechnisms in the distinction between LGOC and HGOC will be available for you to read.

Also the emphasis has turned to primary preventive strategies due to the limited efficacy of current screening methods, especially for women who are at risk of developing epithelial ovarian cancer (EOC). Given that many high-grade serous EOC cases originate primarily in the fallopian tubes, the preventive impact of salpingectomy on EOC incidence is both biologically tenable and solidly supported. You will also have the opportunity to read a meta-analysis evaluating EOC incidence rates associated with salpingectomy, with an exploratory assessment of temporal trends following guideline-driven increases in opportunistic salpingectomy.

Dear Participants,

I would also like to remind you about the seventh Social Responsibility Project organized by the Turkish Ger-man Gynecological Education and Research Foundation (TGGF), scheduled for June 5-6, 2026, in Van-Türkiye. This initiative, taking place in this beautiful city, is traditionally structured into four steps: a public awareness meeting involving local community members, a scientific conference attended by health professionals, the execu-tion of advanced surgeries and medical examinations/screenings for local women, and ultimately, a donation of medical equipment to a local hospital. We believe that our project will be deemed successful if we can prevent even a single maternal death. It is these small efforts that may ultimately pave the way for a significant change. We would be delighted to have our colleagues participate in this intensive scientific endeavor.

Please also take note of the dates "21-25 April 2027" in your calendars for the 16th Turkish German Gynecology Congress which will be held in Antalya.

Dear Esteemed Readers, Authors and Reviewers,

In 2025, we had over 197 article submissions, and we have already published over 47 of them. Numerous obstetrics and gynecological subjects are covered in the published papers. We would like to take this oppurtunity to thank everyone who contributed to our journal last year. We are grateful to our readers, authors, and reviewers. Visit us online at www.jtgga.org, and follow us on Twitter at @ JtggaOfficial to stay in contact.

We are looking forward to receiving your valuable submissions, thank you in advance for your contributions.

Sincerely,

Prof. Cihat Ünlü, M.D.
Editor in Chief of *J Turk Ger Gynecol Assoc*
President of TGGF

Maternal and fetal neudesin levels in pregnancy: associations with gestational diabetes mellitus and pregnancy outcomes

Aya I. Moussa[1], Ahmed W. Morad[2], Nahla A. Nosair[3], Mostafa F. Ellakany[1], Ahmed Soliman[4], Ahmed Fathy[1]

[1]Department of Obstetrics and Gynecology, Kafr Elsheikh University Faculty of Medicine, Kafr Elsheikh, Egypt
[2]Department of Obstetrics and Gynecology, Benha University Faculty of Medicine, Benha, Egypt
[3]Department of Clinical Pathology, Kafr Elsheikh University Faculty of Medicine, Kafr Elsheikh, Egypt
[4]Mansoura University Faculty of Medicine, Mansoura, Egypt

Abstract

Objective: Gestational diabetes mellitus (GDM) is significantly associated with short- and long-term adverse maternal and perinatal outcomes. Despite the important role of neudesin in modulating glucose metabolism and insulin sensitivity, extant literature is scarce on the link between neudesin and GDM. This study investigated neudesin in GDM and its potential as a diagnostic marker and future therapeutic target.

Material and Methods: We conducted a case control study at our department. Forty-five Pregnant women with GDM were included in the study group, while an equal number of healthy pregnancies constituted the controls. The enzyme-linked immunosorbent assay technique was employed to quantify the concentration of neudesin in both maternal and cord samples.

Results: Women with GDM (n=45) exhibited significantly higher maternal and umbilical cord serum neudesin levels compared to controls (median maternal neudesin: 4.9 ng/mL vs. 1.9 ng/mL; median umbilical cord neudesin: 2.6 ng/mL vs. 1.2 ng/mL). Maternal neudesin levels correlated positively with body mass index, fasting insulin, measures of insulin resistance, and neonatal birth weight and inversely with APGAR scores. A maternal neudesin cut-off of 5.25 ng/mL demonstrated high diagnostic accuracy for GDM (area under the curve =0.967, 91.1% sensitivity, 93.3% specificity, 92.2% accuracy).

Conclusion: Neudesin may serve as a potential diagnostic tool for GDM. Future investigations into neudesin as a novel GDM biomarker and potential therapeutic target are urgently warranted.

Keywords: Gestational diabetes mellitus, neudesin, pregnancy, diagnostic biomarker

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Introduction

Gestational diabetes mellitus (GDM) refers to any form of glucose intolerance that develops during pregnancy (1). According to the international diabetes federation, GDM affects approximately 12% of pregnancies worldwide, impacting roughly 20 million births annually (2). In the eastern mediterranean region, the prevalence of GDM is higher, reaching 14.5%, perhaps due to a pre-existing higher prevalence of diabetes mellitus in the region (2). Women with GDM face an increased risk of complications, including gestational hypertension, preeclampsia, prolonged labor, cesarean delivery, and macrosomic babies. These complications may have significant health consequences for both mother and child (3).

Address for Correspondence: *Ahmed Soliman*
e-mail: *ahmedsolimann9090@gmail.com* ***ORCID:*** *orcid.org/0000-0003-3964-3778*

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Several mechanisms may explain the pathophysiological process behind GDM (3-5). Insulin resistance (IR), a natural physiological change during pregnancy, plays an important role in the development of GDM. IR is influenced by factors such as placental hormone production, maternal obesity, and inflammation (3). Moreover, IR intensifies as pregnancy progresses in certain expectant individuals, particularly those with pre-existing obesity and ir (4). Consequently, pancreatic beta cells are unable to maintain normoglycemia by increasing insulin production. The onset of GDM is indicated by the development of aberrant blood glucose levels as a result of this failure (6). Following the establishment of the placenta, the regulatory impact of a variety of signaling molecules, including proteins (including cytokines, growth factors, and glycoproteins), hormones, and other molecules, begins to affect IR (7).

Neudesin, a protein involved in various biological systems, is thought to play a role in regulating glucose metabolism and insulin sensitivity (8). Neudesin has the potential to function as a modulative agent within the regulatory frameworks that govern glucose metabolism and the modulation of insulin responsiveness, as evidenced by research conducted in pediatric cohorts with type one diabetes mellitus and adult populations with type two diabetes mellitus (9). GDM is an aberrant example of carbohydrate metabolic decompensation, and neudesin is purported to play a critical regulatory function in carbohydrate metabolism and energy (10).

The objective of this study was to investigate the levels of neudesin in the blood of pregnant women with GDM and their babies and compare this with pregnancies unaffected by GDM, and thereby shed light on the potential role of neudesin in the development and progression of GDM.

Material and Methods

Study design and approval

From June 2023 to September 2024, we conducted a case-control study on patients of the Department of Obstetrics and Gynecology in Kafr El-Sheikh University Hospital. The study protocol was approved by the Mansoura Faculty of Medicine Mansoura University Ethics Committee (approval number: 20.05.2573, date: 02.05.2023).

Patient selection and enrollment

Participation was voluntary for pregnant women, aged 18-35 years, in whom pregnancies were complicated with GDM, and they were not treated with insulin. Exclusion criteria were: existence of maternal comorbid conditions, including chronic hypertension; pregestational diabetes mellitus; preeclampsia; and chronic renal disease. Further exclusion criteria included the presence of confirmed fetal structural or chromosomal abnormalities. After obtaining informed consent, full history taking was conducted for all participants, including personal, medical, obstetric, and gynecological histories.

GDM ascertainment

GDM was diagnosed by administering a 75-gram oral glucose tolerance test (OGTT) between 24 and 28 weeks of gestation. GDM was diagnosed when one or more abnormal plasma glucose values (fasting >92 mg/dL; one hour >180 mg/dL; and/or two hours >153 mg/dL) were obtained using the criteria of The International Association of Diabetes and Pregnancy Study Groups (11).

Maternal and fetal neudesin ascertainment

Following a 12-hour fast, maternal venous blood samples were drawn from all study participants on the day of OGTT screening. Maternal serum was evaluated for neudesin concentration, fasting glucose and insulin levels and homeostatic model assessment for insulin resistance (HOMA-IR) was calculated. HOMA-IR was calculated using the standard formula: fasting glucose (mmol/L)_x fasting insulin (IU/mL)/22.5 (12). In addition, umbilical venous blood samples were obtained at delivery and fetal neudesin concentrations were evaluated. An enzyme-linked immunosorbent assay (ELISA) technique was used to quantify the concentration of neudesin in maternal and cord samples. We utilized the human neudesin neurotrophic factor ELISA kits (catalog number: 201-12-7672), which were maintained at a temperature of 2-8 °C. The test's sensitivity was 0.073 ng/mL, and the assay range was 0.08 to 20 ng/mL.

Statistical analysis

Statistical analyses were performed using SPSS, version 28 (IBM Inc., Armonk, NY, USA). Normality was assessed with the Shapiro-Wilk test. Descriptive statistics included means/SDs and medians/ interquartile range (IQRs), as appropriate. For non-parametric comparisons, the Mann-Whitney U test was used while for parametric comparisons, the independent samples t-test was employed. Receiver operating characteristic curves determined optimal cut-off values for diagnostic parameters. The Spearman rank correlation coefficient was used to assess relationships between variables. Binary logistic regression identified independent risk factors. statistical significance was defined as $p<0.05$ and high significance as $p\leq0.001$. Maternal age, body mass index (BMI), parity, family history of diabetes, fasting blood glucose levels, and HbA1c were identified as potential confounding variables in the multivariate regression analysis of predictors for GDM. After adjusting for the covariates, maternal serum neudesin levels were identified as a significant independent predictor of GDM risk.

Sample size calculation

A post-hoc power analysis was conducted based on the primary outcome of interest: the difference in median maternal serum neudesin levels between the GDM and control groups. Based on the observed medians (GDM: 4.9 ng/mL vs. control: 1.9 ng/mL) and the IQRs, our sample size of 90 patients provided a high statistical power (>99%) to detect the substantial difference in neudesin concentration at a significance level (α) of 0.05 (two-sided test).

Results

Patients' baseline characteristics

Ninety patients were recruited to the study, 45 in the GDM group and 45 controls. Figure 1 shows the flow diagram of the final included study participants. The mean BMI for women with GDM was 29.2±4.55 kg/m^2, while the mean BMI for the control group was 25.6±6.23 kg/m^2 ($p<0.001$). Gestational age at the study enrollment was similar in both groups. As expected, there were significantly higher levels of fasting insulin and HOMA-IR in women with GDM compared to the control group. The GDM group exhibited a median fasting insulin level of (7-17.5) μU/mL compared to 10 (8-12) μU/mL in the control group. Similarly, median HOMA-IR values were substantially elevated in the GDM group at 3.1 (2.1-4.2) compared to 1 (range: 0.79-1.2) in the control group. Table 1 summarizes the baseline data of the study participants.

Maternal and umbilical cord serum neudesin levels

Compared to controls, we observed significantly elevated neudesin levels in both the maternal serum of women with GDM and umbilical cord samples of fetuses of GDM-complicated pregnancies. The median maternal serum neudesin level was 4.9 (4.45-5.9) ng/mL in the GDM group, considerably higher than the 1.9 (1.65-2.1) ng/mL observed in the control group. Similarly, the median umbilical cord neudesin level was significantly higher in the GDM group at 2.6 (2.1-3.25) ng/mL compared to 1.2 (1.1-1.4) ng/mL in the control group. Table 2 compares GDM-complicated pregnancies and controls according to maternal and umbilical neudesin levels.

Maternal and neonatal outcomes at delivery

Table 3 summarizes the maternal and neonatal outcomes at delivery. Compared to controls, women with GDM had significantly higher BMI at delivery (30.67±4.57 kg/m^2 vs. 27.14±3.23 kg/m^2, $p<0.001$). Women without GDM delivered at a later gestational age than those GDM-complicated pregnancies (38.2±0.41 weeks vs. 37.93±0.5 weeks, $p=0.006$). Furthermore, whereas the neonatal birth weight was significantly later in the GDM group (3455±402 grams vs.

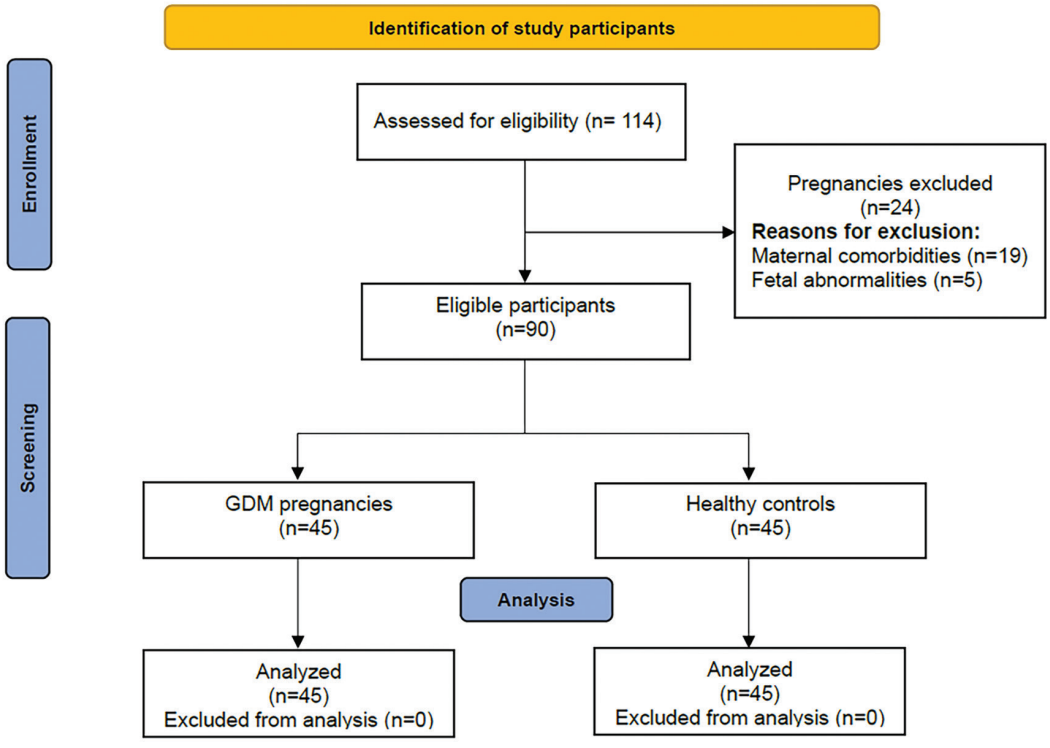

Figure 1. Flow diagram of the final included study participants

Table 1. Comparison between the study groups regarding baseline data

Parameter	GDM group (n=45)	Control group (n=45)	t	p
	Mean ± SD	Mean ± SD		
Age (year)	28.73±5.52	28.13±5.24	0.528	0.598
Gestational age at blood sampling (week)	26.69±1.35	26.58±1.37	0.388	0.699
BMI at sampling	29.18±4.55	25.63±3.23	4.256	<0.001*
Fasting insulin (range)	12 (7-17.5)	10 (8-12)	-1.483	0.138
HOMA-IR (range)	3.1 (2.1-4.2)	1 (0.79-1.2)	-7.948	<0.001*

GDM: Gestational diabetes mellitus, t: Independent sample t-test, SD: Standard deviation, BMI: Body mass index, HOMA-IR: Homeostasis model assessment of insulin resistance. *p<0.05 is statistically significant

Table 2. Comparison between the study groups regarding maternal and umbilical serum neudesin levels

Neudesin (ng/mL)	GDM group (n=45)	Control group (n=45)	z	p
	Median (IQR)	Median (IQR)		
Maternal serum	4.9 (4.45-5.9)	1.9 (1.65-2.1)	-7.655	<0.001*
Umbilical cord	2.6 (2.1-3.25)	1.2 (1.1-1.4)	-7.25	0.006*

IQR: Interquartile range, ng/mL: Nanograms per milliliter, GDM: Gestational diabetes mellitus. *p<0.05 is statistically significant

Table 3. Comparison between the study groups regarding maternal and neonatal outcomes

Parameter	GDM group	Control group	t	p
	Mean ± SD	Mean ± SD		
BMI at delivery	30.67±4.57	27.14±3.23	4.243	<0.001*
Gestational age at delivery (week)	37.93±0.5	38.2±0.41	-2.797	0.006*
Birth weight (gm)	3455±402	3151±167	4.684	<0.001*
APGAR at 1 minute	8.07±0.48	8.91±0.29	-10.652	<0.001*
APGAR at 5 minutes	9.02±0.45	9.91±0.29	-11.133	<0.001*

APGAR: Appearance, pulse, grimace, activity, and respiration, GDM: Gestational diabetes mellitus, SD: Standard deviation, t: Independent sample t-test, *p<0.05 is statistically significant

3151±167 grams, p<0.001), APGAR scores at 1 and 5 minutes were significantly higher for neonates delivered to women in the control group [(8.91±0.29, and 9.91±0.29) vs. (8.07±0.48, 9.02±0.45), p<0.001], respectively.

Correlation between neudesin levels and participants' baseline characteristics

Significant positive correlations were observed between maternal neudesin levels and BMI, both at sampling (r=0.377) and delivery (r=0.361) as well as fasting insulin levels (r=0.215), and HOMA-IR (r=0.782) indices. Neonatal birth weight was positively correlated with maternal serum neudesin level (r=0.213), and umbilical cord neudesin level (r=0.209). Both APGAR scores at 1 and 5 minutes after delivery were significantly correlated with maternal serum neudesin level (r=0.61, r=0.65, respectively), and umbilical cord serum neudesin level (r=0.55, r=0.57, respectively). Tables 4 and 5 show the correlations between neudesin levels and participants' characteristics at baseline and delivery.

Performance of neudesin levels in GDM diagnosis

We further investigated the diagnostic potential of maternal and umbilical cord neudesin levels for GDM. For maternal serum neudesin, a cut-off value of 5.25 ng/mL or higher demonstrated high diagnostic accuracy, with an area under the curve (AUC) of 0.967 (Figure 2). This translated to 91.1% sensitivity, 93.3% specificity, and an overall accuracy of 92.2% (Supplementary Table 1). Further, the umbilical cord neudesin, with a cut-off value of 3.85 ng/mL or higher, showed promising results. It exhibited a sensitivity of 95.6%, a specificity of 86.7%, and an AUC of 0.942 (Figure 3). The overall accuracy was 91.1% (Supplementary Table 2). Finally multivariate regression analysis demonstrated that maternal serum neudesin levels were independently associated with a 620-fold risk of GDM (Supplementary Table 3).

Table 4. Correlation between maternal, neonatal neudesin and baseline participants' characteristics

Variable	Maternal serum		Umbilical cord	
	r	p	r	p
Age (year)	-0.017	0.847	0.037	0.728
Gestational age at sampling	-0.304	0.004*	-0.341	0.001*
BMI at sampling	0.377	<0.001*	0.299	0.004*
Fasting insulin	0.215	0.041*	0.296	0.005*
HOMA-IR	0.782	<0.001*	0.774	<0.001*
Maternal serum neudesin	-	-	0.92	<0.001*
Umbilical cord neudesin	0.92	<0.001*	-	-

HOMA-IR: Homeostasis model assessment of insulin resistance, r: Spearman rank correlation coefficient, BMI: Body mass index. *p<0.05 is statistically significant

Table 5. Correlation between maternal neudesin and delivery outcomes

Variable	Maternal serum		Umbilical cord	
	r	p	r	p
BMI at delivery	0.361	<0.001*	0.29	0.006*
Gestational age at delivery (week)	-0.216	0.041*	-0.187	0.078
Birth weight (gm)	0.213	0.044*	0.209	0.049*
APGAR at 1 minute	-0.615	<0.001*	-0.552	<0.001*
APGAR at 5 minutes	-0.65	<0.001*	-0.577	<0.001*

APGAR: Appearance, pulse, grimace, activity, and respiration, r: Spearman rank correlation coefficient, BMI: Body mass index, *p<0.05 is statistically significant

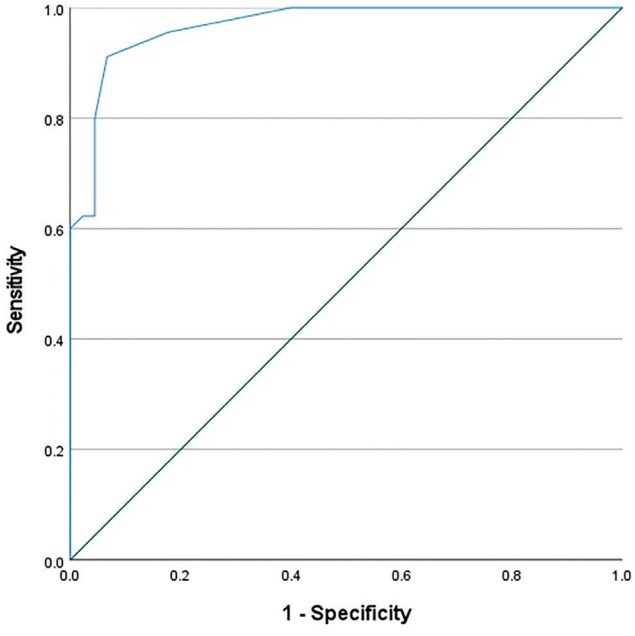

Figure 2. Receiver operating characteristic curve showing performance of maternal neudesin in diagnosis of gestational diabetes

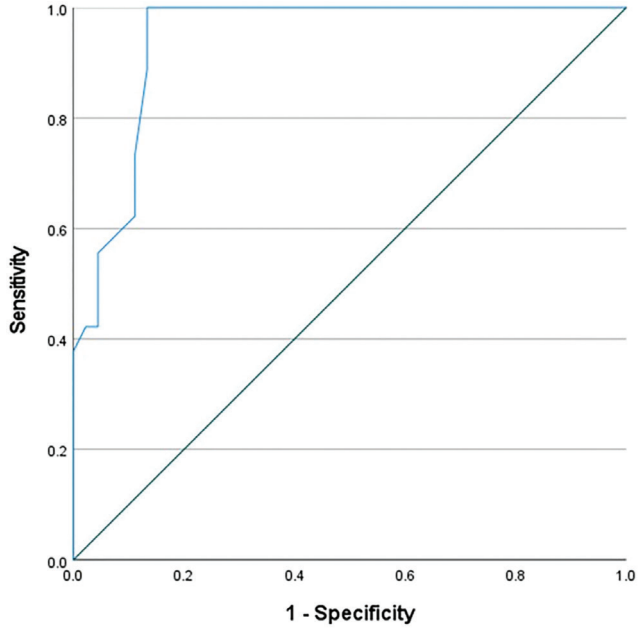

Figure 3. Receiver operating characteristic curve showing performance of umbilical cord neudesin in diagnosis of gestational diabetes

Discussion

Findings summary

This study investigated associations between neudesin and GDM. Women with GDM exhibited higher maternal and umbilical cord neudesin levels, higher BMI, fasting insulin, and HOMA-IR compared to controls. Elevated neudesin levels correlated with adverse pregnancy outcomes, including higher birth weight and lower APGAR scores in newborns. Importantly, maternal serum neudesin demonstrated high diagnostic accuracy for GDM, with high sensitivity, specificity, and AUC. These findings suggest that neudesin may serve as a valuable biomarker for GDM diagnosis and could potentially aid in identifying women at increased risk for adverse pregnancy outcomes.

Our findings in the context of previous literature

Our findings are consistent with previous reports that implicated high BMI as a potent risk factor for GDM (13,14). We also found elevated fasting insulin and HOMA-IR levels in women with GDM, consistent with studies by Paracha et al. (15) and Adam et al. (4). with a larger sample size, our current study complements and extends earlier reports by Eren et al. (10) on significantly higher maternal serum and umbilical cord neudesin levels in pregnancies complicated by GDM.

However, previous reports on the association between neudesin, BMI and GDM are not always consistent. For instance, contrary to our findings, Atalay et al. (16) reported lower neudesin levels in women with GDM. Furthermore, the significantly lower BMI at delivery reported by Eren et al. (10) in women with GDM is at variance with the higher delivery BMI observed in our study and also reported by Atalay et al. (16). This discrepancy may be attributable to differences in the study populations, particularly the baseline prevalence of obesity or ethnic-specific variations in metabolic risk factors, given the higher baseline prevalence of diabetes in the eastern mediterranean region. Our study population, characterized by generally high BMI, elevated fasting insulin, and high HOMA-IR in the GDM group, may represent a cohort where GDM development was strongly driven by pre-existing obesity and pre-pregnancy IR which worsened further during pregnancy. The significant positive correlation we observed between maternal neudesin levels and BMI, fasting insulin, and HOMA-IR (with r=0.782 for HOMA-IR) suggests that in this specific, higher-BMI population, neudesin elevation is closely tied to the degree of metabolic dysfunction and IR. These variations highlight the complex relationship between neudesin levels, BMI, and GDM.

In terms of delivery outcomes, our findings are consistent with previous reports (17-19), demonstrating significantly lower gestational ages at delivery, higher birth weights, and lower APGAR scores in neonates of women with GDM-complicated pregnancies. In contrast, although Eren et al. (10) reported lower APGAR scores in neonates of women with gdm-complicated pregnancies, they found no significant differences in gestational weight at delivery or birth weight. The adverse outcomes in our cohort suggest that women with GDM in the present study may have experienced a greater degree of uncontrolled maternal hyperglycemia or more severe underlying placental dysfunction compared to the populations in the contrasting studies. The weak positive correlation between maternal and umbilical cord neudesin levels and neonatal birth weight (r=0.213 and r=0.209, respectively), and the negative correlation with APGAR scores highlight a potential role for neudesin as both a diagnostic biomarker and as a mechanistic link in the placental-fetal programming that leads to macrosomia and fetal distress. Further research is warranted to elucidate the specific mechanisms by which neudesin influences fetal growth and development in the context of GDM pathophysiology.

Study limitations

Our findings should be interpreted in the context of the following limitations: first, we could not conduct repeated measures of maternal and umbilical cord neudesin levels due to logistical constraints. Second, since GDM diagnosis preceded neudesin evaluation, we could not ascertain a temporal biological sequence of events between GDM and neudesin. The small sample size (n=90), the single-center, and the case-control design all restrict the generalizability of the findings. Furthermore, the study lacks long-term follow-up to assess the postnatal metabolic status of mothers or the long-term health outcomes of the infants. Finally, the absence of a formal pre-study power calculation may be seen as a methodological limitation but the post-hoc analysis confirmed that the study was sufficiently powered to detect the substantial difference in neudesin levels observed between the groups.

Conclusion

This study provides compelling evidence that neudesin may be independently associated with GDM. Elevated neudesin levels in maternal and umbilical cord serum and strong correlations with key metabolic parameters and neonatal outcomes highlight its potential as a valuable diagnostic marker. The high diagnostic accuracy achieved with specific neudesin cut-offs suggests its potential clinical utility in GDM screening and risk assessment. Furthermore, the substantial increase in GDM risk associated with elevated maternal neudesin levels underscores its potential pathophysiological role. Future research should focus on elucidating the underlying mechanisms by which neudesin contributes to gdm and exploring its potential as a therapeutic target.

Ethics

Ethics Committee Approval: *The study protocol was approved by the Mansoura Faculty of Medicine Mansoura University Ethics Committee (approval number: 20.05.2573, date: 02.05.2023).*

Informed Consent: *All study particpants gave informed written consent.*

Footnotes

Author Contributions: *Surgical and Medical Practices: A.W.M., N.A.N., Concept: A.I.M., Design: A.F., M.F.E., Data Collection or Processing: A.F., A.S., Analysis or Interpretation: A.S., M.F.E., Literature Search: N.A.N., A.I.M., Writing: A.I.M., A.W.M., N.A.N., A.F., A.S.*

Conflict of Interest: *No conflict of interest is declared by the authors.*

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Supplementary File Tables: https://d2v96fxpocvxx.cloudfront.net/17d041fe-057c-4c8e-a75d-948e33c0152a/content-images/7cba4a2e-c6f1-4ab1-972f-2f4c629b1611.pdf

Is first trimester screening an opportunity for early diagnosis of structural anomalies?: A retrospective cohort study

Talat Umut Kutlu Dilek[1], Eren Kaya[2], Elif Aygün[3], Özlem Pata[4]

[1]Department of Perinatology, Başkent University İstanbul Hospital, İstanbul, Türkiye
[2]Clinic of Obstetrics and Gynecology, Malazgirt State Hospital, Muş, Türkiye
[3]Department of Obstetrics and Gynecology, Acıbadem University Atakent Hospital, İstanbul, Türkiye
[4]Department of Perinatology, Acıbadem University Faculty of Medicine, İstanbul, Türkiye

Abstract

Objective: To share our experience of ultrasonographic evaluation of fetal anatomy in the first trimester and pregnancy follow-up in a tertiary center.

Material and Methods: This retrospective study was conducted in the Acıbadem University Atakent Hospital and Acıbadem University Bakırköy Hospital Prenatal Diagnosis Units between April 2015 and December 2019. The study group included pregnant women referred for first-trimester aneuploidy screening and anomaly survey.

Results: The mean maternal age was 31.28±4.43 years and ranged from 20-49 years. The median gestational week at which first-trimester evaluation was made was 12.4 weeks. Of 3254 cases, 55 (1.69%) had pathologic ultrasound findings in the first-trimester anomaly scan, including increased nuchal translucency (NT) value over 95th percentile in 34 fetuses (52.3%) with structural anomaly. Median (range) crown-rump length was 58.69 (45-83) mm, and the median NT value was 3,5 (1.5-12) mm for fetuses with abnormal sonographic findings. The total detection rate for sonographic anomalies in the first-trimester scan was 60.43%. Of note, 27.3% of fetuses with detected anomalies had multiple congenital anomalies. Twenty-four new cases were diagnosed in the second trimester, and 11 new cases were detected in the last trimester from the same cohort.

Conclusion: Screening between 11-14 weeks of pregnancy may be an opportunity to evaluate maternal health and detect severe fetal anomalies. The family should be counselled about structural anomalies that may be detected later, especially in the second and third trimesters, the limitations of the technique, and the ongoing progress of fetal development. [J Turk Ger Gynecol Assoc. 2026; 27(1): 8-18]

Keywords: Prenatal diagnosis, anomaly, fetus, prenatal ultrasound

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Introduction

A newly diagnosed pregnancy has a 2-3% risk of significant structural anomaly (1). The primary imaging tool to screen and diagnose structural anomalies is ultrasound and this technique has been included in all antenatal follow-up programs for 30 years. Early detection of fetal anomalies is an opportunity to diagnose comorbid genetic problems, counsel the family, and evaluate the relevance of prenatal or postnatal treatment. The earlier diagnosis of such anomalies, which are incompatible

Address for Correspondence: *Eren Kaya*
e-mail: *erenkaya@ku.edu.tr* ***ORCID:*** *orcid.org/0000-0003-4479-7317*

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with life, provides parents with the option to terminate the pregnancy. The early procedure is usually safer and more acceptable for the parents. Ultrasound screening for fetal structural anomalies should be performed during pregnancy, as part of the examination during first-trimester aneuploidy screening, or in the second trimester between the 18 and 23 weeks of pregnancy. The second-trimester examination is now mandatory in the prenatal surveillance protocol in many countries. Factors affecting the prenatal diagnosis of fetal anomalies include technique, gestational age at which the examination was performed, whether the screened population is low or high-risk, the woman's body mass index (BMI), the practitioner's experience, and the region of interest (2). The detection rate of fetal anomalies increases in pregnant women with known risk factors for fetal anomalies (3). Moreover,the rate of fetal anomaly diagnosis in reference centers was reported to be 2.7 times higher than in centers that provide standard care in the RADIUS study (4). A first-trimester screening test has been performed more frequently since the end of the 1990s and simultaneously, an examination of fetal anatomy was focused on by various centers. Thus, the technical and theoretical infrastructure for anatomical examination was developed gradually. Both the International Society of Ultrasound in Obstetrics and Gynecology (ISUOG) and the American Institute of Ultrasound Medicine have set out the criteria for screening for this period (5-7).

A comprehensive first-trimester ultrasound scan, performed between 11+0 and 14+0 weeks' gestation, involves a systematic assessment to ensure accurate and thorough fetal evaluation (7). Key steps include confirming fetal viability and determining gestational age through crown–rump length (CRL) measurement. The scan should assess the number of fetuses, chorionicity in multiple pregnancies, and screen for aneuploidies by measuring nuchal translucency (NT) and evaluating the nasal bone, ductus venosus flow, and tricuspid valve regurgitation. A detailed anatomical survey should follow, covering the head, brain, face, neck, thorax, heart, abdomen, spine, limbs, and genitalia, using both transabdominal and, when necessary, transvaginal approaches for optimal visualization. Doppler studies of the uterine arteries can also be incorporated to screen for pre-eclampsia. The use of high-quality ultrasound equipment and adherence to safety standards, including the As Low As Reasonably Achievable (ALARA) principle, are essential to ensure the effectiveness and safety of the scan.

While 35-40% of fetal anomalies can be detected with fetal anatomical examinations performed at the end of the first trimester in the low-risk pregnant population, this detection rate can be as high as 60% in the high-risk group (8). Primary requirements for first-trimester anatomy exams are experienced practitioners and high-frequency transvaginal, linear, or convex abdominal probes (9-11). Detection rates of fetal anomalies were higher in the lethal or multiple congenital anomalies. The primary imaging tool to screen and diagnose structural anomalies is ultrasound. Consequently, this technique has been in all antenatal follow-up programs for 30 years. Early detection of fetal anomalies is an opportunity to diagnose comorbid genetic problems, counsel the family, and evaluate the relevance of prenatal or postnatal treatment. The earlier diagnosis of such anomalies, which are incompatible with life, allows parents to opt for termination of pregnancy. The early procedure is usually safer and more acceptable for the parents. Ultrasound screening for fetal structural anomalies could be performed during pregnancy, as part of the examination during first-trimester aneuploidy screening, or in the second trimester between the 18 and 23 weeks of pregnancy (2). History of previous surgery, high BMI, number of fetuses, and fetal position are among the factors that affect the examination's success and the detection rate of fetal anomalies.

Transvaginal evaluation between 11th and 12th gestational weeks provides an excellent opportunity to evaluate fetal structures. However, despite the better resolution of using a high-frequency transvaginal transducer close to the fetus, the limited flexibility and the inability to obtain different examination planes are well-known handicaps of transvaginal exams in the late first trimester. The safety of the fetus (and mother) are paramount and should be foremost when planning any ultrasonographic evaluation. The ALARA principle is also valid for first-trimester examinations. While B-mode and M-mode can be safely applied throughout the pregnancy, pulse Doppler should be used only for limited periods and in indicated cases due to its thermal effect on the developing embryo and fetus (12). Reducing the acoustic output will lower the thermal index (TI) without affecting the resolution (13). In this retrospective study, we share our experience from a tertiary center about ultrasonographic evaluation of fetal anatomy in the first trimester and pregnancy follow-up.

Material and Methods

This retrospective study was conducted in Acıbadem University Atakent Hospital and Acıbadem University Bakırköy Hospital Prenatal Diagnosis Units between April 2015 and December 2019. This study was approved by the Acıbadem University Ethics Commitee (approval number: ATADEK 2021-02/01, date: 28.01.2021). Both maternal-fetal medicine units provide early anatomy surveys to high-risk pregnancies, such as a history of previous fetal anomaly, multiple pregnancies, pregnancies with a high-risk first-trimester aneuploidy screening test, high-risk cell-free DNA test, maternal teratogen exposure, and consanguineous couples with a history of genetic disease.

The study group included pregnant women referred for first-trimester aneuploidy screening and anomaly survey. Of these, 3254 cases had follow-up data, including second-trimester examination and pregnancy outcome data. Cases that had no second-trimester examination, *in-utero* demise, higher-order multiples, and pregnancies lost from pregnancy follow-up were excluded from the study. All first and second-trimester exams were performed by the same maternal-fetal medicine specialists (TUKD and ÖP) using Voluson E8 Expert, equipped with RMC-6 or C1-5 D convex probe or an E10, equipped with a C4-8 convex probe (General Electric, Chicago, IL, USA). The RIC6-12 transvaginal probe was used in selected cases for transvaginal exam. Both operators had more than 10 years of experience in first-trimester ultrasound practice. All ultrasound examinations were performed supine and with an empty bladder. The average total examination time for the first-trimester examination was 45 minutes (including NT, nasal bone, ductus venosus and tricuspid regurgitation, fetal anatomy exam, and bilateral uterine artery Doppler measurements).

In cases of inappropriate fetal position during the 30-minute examination period, the examination was stopped and repeated following a one-hour break. The region of interest was magnified to fill 70% of the screen. The scan area was narrowed to 20-30 degrees for a better frame rate, and harmonics were adjusted in the middle. To follow the ALARA principle, ultrasound acoustic output settings were set to yield a TI value below 0.5 in the study area. Our checklist was based on a modified ISUOG guideline (Table 1 and Figures 1, 2). In case of cardiac anomaly suspicion or abnormal cardiac examination findings, fetal echocardiography was done by an experienced pediatric cardiologist with expertise in fetal cardiology. In addition, follow-up exams and postnatal echocardiography were performed to ascertain cardiac anomalies. Genetic and prognostic counseling was provided by a medical genetics specialist, pediatric cardiologist, and maternal-fetal medicine specialist.

The first-trimester examination was performed between the 11th and 14th weeks of gestation (CRL 45 mm to 84 mm).

Table 1. Checklist for the first trimester exam

Region	Examination plane	Details
Head	Transverse-sagittal	Cranial bones Midline falx Choroid plexus-filled ventricles Fourth ventricle, intracranial translucency Midbrain
Neck	Sagittal	Nuchal translucency
Face	Transverse-sagittal-coronal	Eyes with pair of lenses Nasal bone Profile view with palate Retronasal triangle
Chest and heart	Transverse	Symmetrical lung fields Four symmetrical chambers** Outflow tracts* Three-vessel trachea view* Rule out effusions and masses
Abdomen	Transverse-sagittal-coronal	Stomach (fluid filled and left upper quadrant location) Urinary bladder Kidneys
Abdominal wall	Transverse-sagittal	Normal cord insertion Rule out abdominal wall hernias
Extremities		Four limbs each with three symmetrical segment Hand and feets with normal orientation
Spine	Sagittal	Vertebra and intact overlying skin
Placenta	-----	Size, location, proximity to cervical os
Uterine Arteries	-----	Bilateral uterine artery pulsatility index
Umbilical Cord	Transverse	Three-vessel cord

*Evaluated by high-definition power Doppler flow
**Evaluated by both grayscale and high-definition power Doppler flow

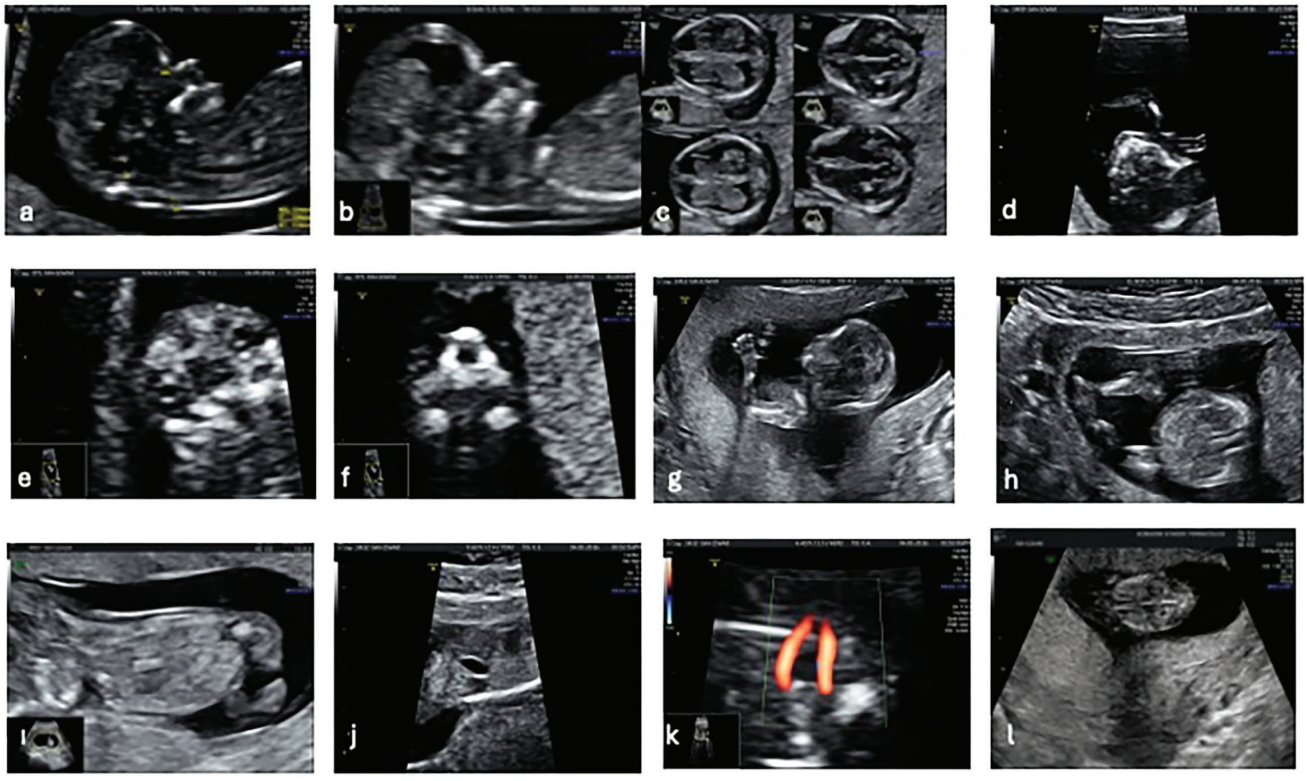

Figure 1. Standard sonographic planes for anatomical survey in the first-trimester exam. Fetal profile view to measure NT (a), fetal profile in the mid-sagittal view (b), axial planes of fetal cranium (c) , axial view of hard palate (d), bilateral orbita and lenses (e), retronasal triangle (f), upper extremity, hand, and fingers (g), lower extremity and foot (h), skin overlying fetal spine (i), fetal stomach (j), fetal bladder and bilateral umbilical arteries (k), bilateral kidneys (l)
NT: Nuchal translucency

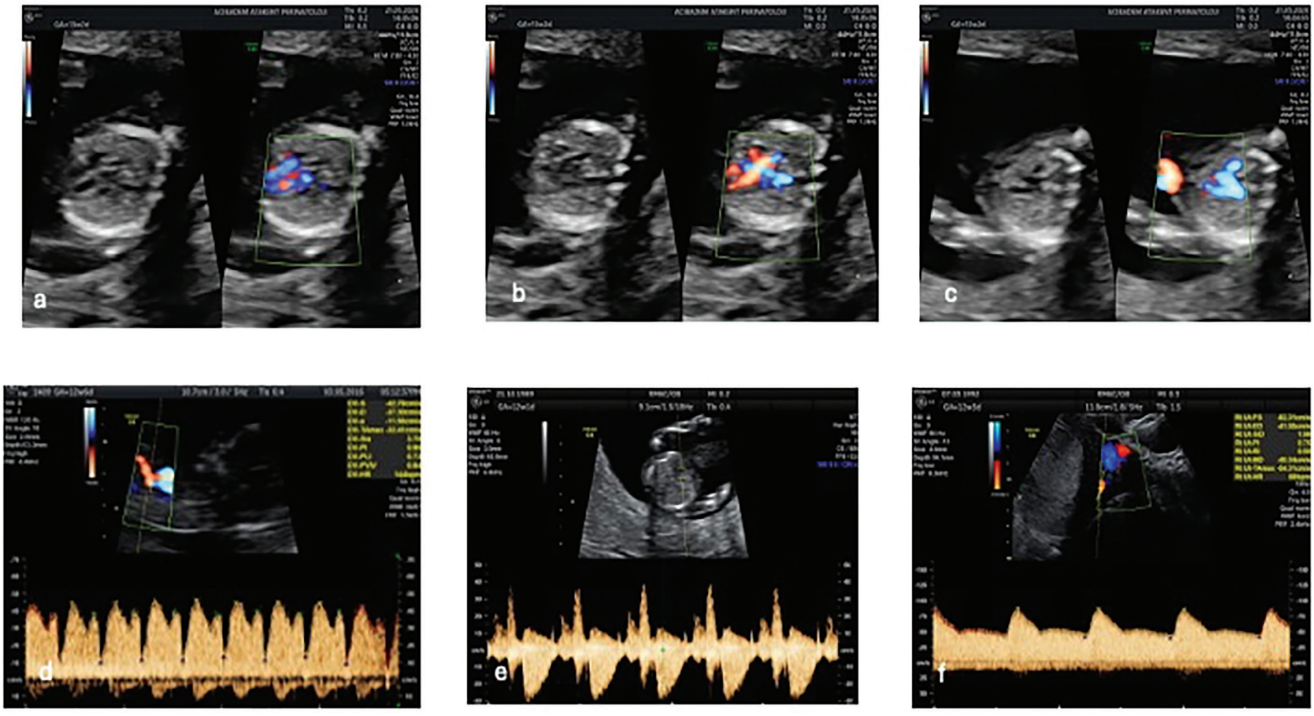

Figure 2. Standard sonographic planes for heart exam and Doppler exam in the first-trimester exam (with high definition flow Doppler). 4-chamber view (a), crossing of great vessels (b), three-vessel view (c), triphasic flow of Ductus venosus (d), assessment of tricuspid valve regurgitation (e), uterine artery Doppler (f)

Before the ultrasound examination, maternal characteristics were recorded including medical history, BMI, and mean arterial pressure. NT was measured in the midsagittal section under appropriate magnification, and the head and one third of the fetal thorax filled the screen. The largest NT value from three consecutive measurements was used for the combined aneuploidy screening test. An invasive genetic test (Chorionic Villus Sampling or amniocentesis) was suggested in case of NT measurement over 3.5 mm (99th percentile) or the existence of structural anomaly without performing a screening test. Women were informed about an estimated individual risk for trisomy 13, 18, and 21. We suggested an invasive genetic test for pregnancies with significant structural abnormalities and those who had a high-risk screening test for aneuploidy for fetal karyotyping. Maternal cell-free DNA testing for trisomies 21, 18, and 13 was suggested for cases with moderate risk (combined trisomy 21 risk was between 1/51 and 1/1000) or parental request.

Termination of pregnancy was offered in case of co-existing multiple structural anomalies, lethal anomalies, and anomalies with co-existing aneuploidies by decision of the local expert committee (including an obstetrician, maternal-fetal-medicine specialist, and pediatric cardiologist or pediatric surgeon). Fetal anatomy was re-examined between 18 and 23 weeks of gestation following an unremarkable first-trimester examination. Soft markers (pyelectasis, echogenic intracardiac focus, choroid plexus cyst) were not considered structural anomalies, and further evaluation was not performed. Genetic counseling and additional diagnostic tests were performed for fetuses with aberrant right subclavian artery, thickened nuchal fold, absence of nasal bone, ventriculomegaly, or grade 3 echogenic bowel.

Statistical analysis

Statistical analysis was performed using SPSS, version 28.0 (IBM Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was used to determine the distribution of continuous data. Comparative analysis between groups used the chi-square test for categorical data, the Mann-Whitney U test, and the Student's t-test for comparing medians and means, respectively.

Results

This retrospective study included 3,254 cases whose pregnancy follow-up data were available, including first and second-trimester examinations. Median maternal age was 31.28±4.43 years and ranged from 20-49 years. Median gestational week at which first-trimester evaluation was made was 12.4 weeks, and median (range) CRL was 64 (45.1-84) mm when the examination was performed. We performed a combined transvaginal and transabdominal approach in 32 cases (1.86%). The median maternal BMI was 24.325 (18-51.8) kg/m^2. Ninety percent of pregnancies were spontaneously conceived. The first trimester anomaly scan identified 55 of 3,254 cases (1.69%) pathologic ultrasound findings, including enlarged NT (including cystic hygromas) with an NT >95th percentile. NT value was >95th percentile in 34 fetuses (52.3%) with structural anomaly when cases with isolated enlarged NT (n=10, 15.38%) were excluded. Twenty-one (32.32%) fetuses with structural anomaly had NT <95th percentile. Moreover, 40 cases had only one, and 15 cases had multiple congenital anomalies (Table 2). Examples from the diagnosed structural anomalies are shown in Figure 3. Median CRL was 58.69 (45-83) mm and median NT value was 3.5 (1.5-12) mm for fetuses with abnormal sonographic findings.

An invasive genetic test was performed in 29 cases (CVS for 19 cases and amniocentesis for 10 cases). Karyotype results are shown in Table 3.

The distribution of fetal anomalies by systems and trimesters is summarized in Table 4. The prevalence of major fetal structural anomaly was 1.69% (55/3254) for the first-trimester exam, including high-risk pregnancies, and 0.74 % (24/3210) for the second-trimester exam. However, following the exclusion of cases with isolated increased NT (NT >95th percentile), these proportions were 1.38% for the first trimester and 0.72% for the second trimester, respectively. The total detection rate for the first trimester scan was 60.4%. Nearly half of the detected congenital anomalies were congenital heart defects (43.63%). In addition, 27.27% of fetuses with detected anomalies had multiple congenital anomalies. The coexistence of multiple anomalies contributed to earlier diagnosis of fetal defects in the first trimester.

Of the 55 case that were detected in the first trimester, nine (16.4%) were lost follow-up and 35 (63.6%) pregnancies with anomaly underwent termination of pregnancy, including intrauterine demise, selective fetocide, and radiofrequency ablation of co-twin pair in twin reverse arterial perfusion at 16 weeks of pregnancy. Only 16 (29.1%) cases resulted in term delivery, and 5 (9.1%) cases resulted in preterm live birth. A total of four selective fetocide procedures were performed on multiplets with the anomaly in the first trimester by intracardiac potassium chloride, including one fetus with acrania-exencephally in triplets, and three cases with multiple and lethal anomalies including cardiac anomalies. Two cases that resulted in preterm delivery were multiplets. One of these was a monochorionic monoamniotic twin pregnancy, and the twin pair had (corrected) L-type transposition of great arteries. Planned cesarean delivery was performed in this monochorionic twin pregnancy at the 33rd weeks of gestation.

Table 2. Summary of fetal structural anomalies and distribution by trimesters

Fetal structural anomaly	First trimester	Second trimester
Acrania-exencephaly	5	0
Alobar holoprosencephaly**	1	0
Posterior fossa cyst	2	0
Lissencephaly + growth restriction	0	1
Ventricular septal defect (VSD)	5 (one case coexistent with tricuspid atresia	6
Atrioventricular septal defect (AVSD)	7	0
Tetralogy of fallot	2	0
Double outlet right ventricle	1 coexistent with VSD	0
Transposition of great arteries	2* coexistent with VSD	1
Truncus arteriosus	2 coexistent with VSD	
Coarctation of aorta	0	1
Hypoplastic left heart	2	2
Hypoplastic right ventricle	1	0
Pericardial Effusion	0	1
Absence of ductus venosus	1	0
Congenital cystic adenomatoid malformation	0	1
Pleural effusion	1	0
Non-visualization of gallbladder	0	1
Pelvic kidney	0	1
Unilateral renal agenesis	0	1
Abdominal cyst	1	0
Umbilical vein varice	0	1
Cystic hygroma	2	0
Posterior urethral valve	0	1
Bilateral moderate pyelectasis	0	1
Nasal hypoplasia	0	1
Pes equinovarus	0	2
Sacral meningocele	0	1
Unilateral phocomelia	1	0
Sirenomelia	1	0
TRAP	2	0
AVSD + pes equinovarus	1	0
AVSD + maxillary gap + cleft lip and palate**	1	0
VSD + extremity contractures	1	0
VSD + omphalocele	2	0
VSD + omphalocele + holoprosencephaly	1	0
VSD + bilateral cleft lip-palate + omphalocele + polydactyly	1	0
VSD + interrupted aorta	1	0
VSD + posterior fossa cyst + retrognati	1	0
Omphalocele + pleural effusion	1	0
VSD + nasal hypoplasia + strawberry head	0	1
Single ventricle + omphalocele	1	0
VSD + posterior fossa cyst + hypertelorism	1	0
Strawberry head + bilateral clenched hands	1	0
Posterior fossa cyst + hypoplastic left heart	1	0
Pes equinovarus + strawberry head	1	0
Atrial septal defect + unilateral pyelectasis + single umbilical artery	1	0
Total	55	24

*One of the diagnosed cases was defined as D type transposition in the first trimester, second case was evaluated as suspicious cardiac findings in the first trimester and diagnosed as corrected (L type) transposition in the early second trimester
**Retronasal triangle was not visualized
TRAP: Twin reversed arterial perfusion

Twenty-four new cases were diagnosed in the second trimester (Table 5). The median gestational age was 20 weeks for the fetal anomalies detected in the second trimester. One of the fetuses with a hypoplastic left heart (Case 19) was evaluated as suspicious for ventricular asymmetry in the first-trimester exam. Two cases underwent termination of pregnancy, two cases were delivered preterm, and two cases were lost follow-up. In addition, 11 new cases from the same cohort were detected in the last trimester. The median gestational age was 30 weeks for the fetal anomalies that were detected in the third trimester (Table 5). Case 34 had suboptimal sonographic evaluation in both the first and second-trimester exams.

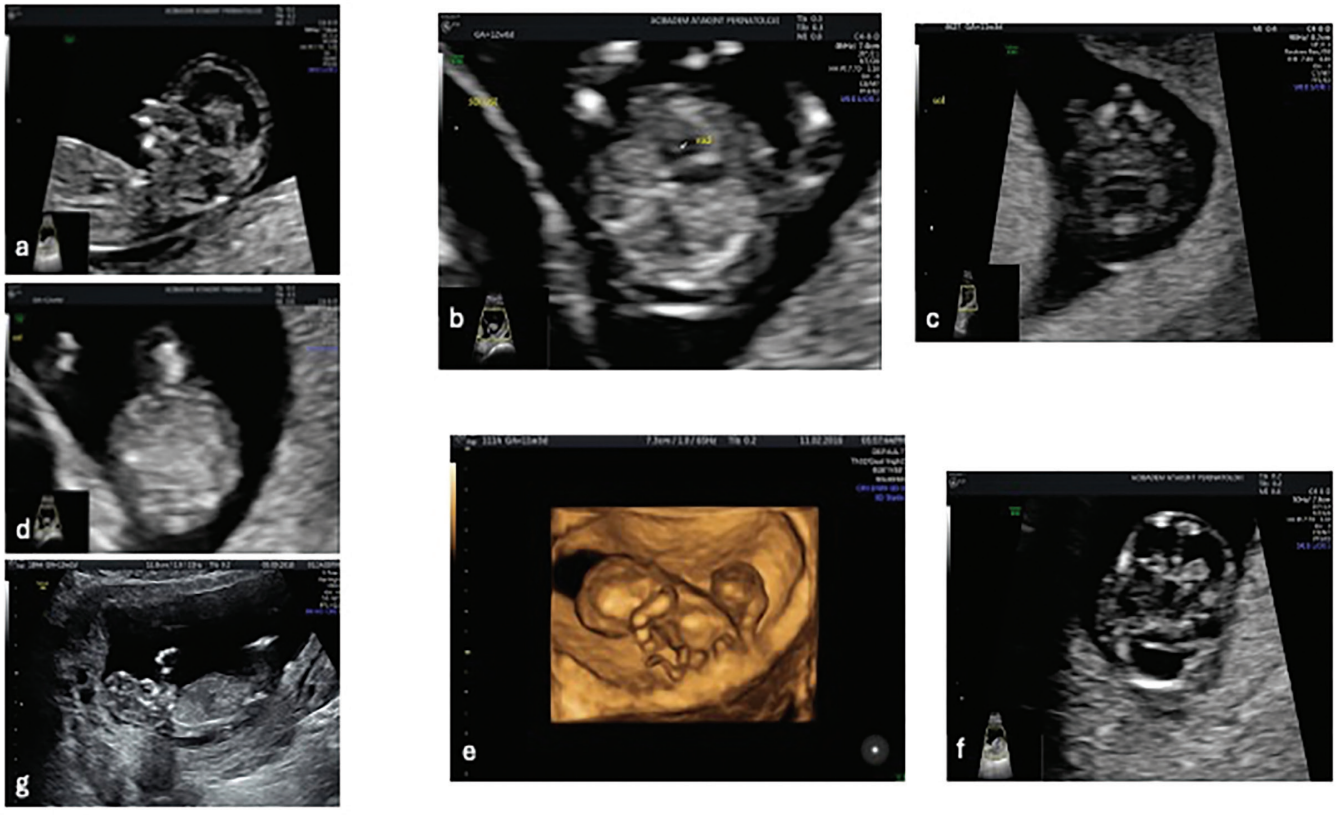

Figure 3. Examples of diagnosed fetal anomalies. Retrognatia and maxillary cleft (a), ventricular septal defect (b), bilateral cleft lip and palate (c), omphalocele (d), acardiac twin pair of monochorionic twin pregnancy (e), posterior fossa cyst (f), acrania (g)

Table 3. Karyotype results of fetuses with congenital anomalies detected in the first trimester which underwent invasive genetic diagnosis in the first/second trimester

Karyotype	n
Partial trisomy 9	1
Trisomy 13	1
Trisomy 18	4
Trisomy 21	6 (including one mosaic case)
Turner syndrome	1
Triploidy	2
Noonan syndrome	1
46; t13;14 (balanced)	1
46; t17;13 (unbalanced)	1
Normal constitutional karyotype	11

Discussion

The first-trimester combined test replaced the second-trimester serum tests to screen for common aneuploidies in the early 1990s (14). High-frequency ultrasound probes, including transvaginal techniques and increased operator experience, contributed to a better and earlier definition of fetal anatomy in the late first trimester. Meanwhile, as a suspicious sign for common trisomies, increased NT was introduced and was also shown to be a warning sign for certain genetic syndromes, congenital cardiac, and non-cardiac anomalies. However, NT has been found within standard reference ranges in 67% of fetuses diagnosed with structural anomalies in the first trimester by gestational week (15) and 95% of fetuses with increased NT had co-existent or multiple fetal anomalies (16). The median NT value of fetuses that had structural anomalies in the first-trimester exam was reported as 2.2 mm in a recent study (17). In the present study, nearly 53% of fetuses with structural anomalies in the first-trimester exam had an NT value above the 95th percentile, and the median NT value was 3.5 mm. In another series, the number of cases above the 95th percentile was reported as 26% (18). Of note, none of the 24 fetuses with anomalies diagnosed in the second-trimester exam had NT >95th percentile.

Following the development of the guidelines and publications about the anatomical survey of the second-trimester fetus, an area of interest is the technical aspects and extent of the

Table 4. Distribution of fetal anomalies by systems.

	1st trimester	2nd trimester	3rd trimester	Total
Central nervous system	8 (14.54 %)	1 (4.16 %)	0	9 (9.89 %)
Face-neck*	2 (3.64)	1 (4.16 %)	1 (8.33)	4 (4.4 %)
Thorax	1 (1.82 %)	1 (4.16 %)	0	2 (2.2 %)
Heart	24 (43.63 %)	11 (45.84)	3 (25 %)	38 (41.75 %)
Alimentary tract-abdomen	1 (1.82 %)	2 (8.34 %)	3 (25 %)	6 (6.59 %)
Genitourinary system	0	4 (16.68 %)	2 (16.67 %)	6 (6.59 %)
Skeleton	2 (3.64 %)	2(8.34 %)	0	4 (4.4 %)
Spine	0	1 (4.16 %)	0	1 (1.1 %)
Multiple anomalies	15 (27.27 %)	1 (4.16 %)	2 (18.18 %)	18 (20.88 %)
TRAP**	2 (3.64 %)	0	0	2 (2.2 %)
Total	55 (60.43 %)	24 (26.37 %)	11 (13.2 %)	90 (100 %)

Data shown as n (%)
*Including cervical cystic hygromas with multiple septations
**Twin reversed arterial perfusion

fetal anatomical examination in the first trimester (19). The fetuses with multiple congenital anomalies or increased NT could be detected earlier; however, it is impossible to diagnose congenital anomalies that may occur later than first trimester due to fetal developmental dynamics during the pregnancy (19,20). Furthermore, overdiagnosed structural anomalies, such as ventricular septal defects, nuchal edema, abdominal cysts, and omphalocele were reported previously (21). Our unpublished data were consistent with previous literature about high false positivity for certain suspicious findings.

Major handicaps of first-trimester anatomic examinations are the size of the fetus, the ongoing fetal development of the central nervous system, alimentary tract, lungs, and late diagnosis of non-lethal skeletal dysplasias. In addition, gestational age at fetal exam, experience of operator, number of fetuses, and patient-related conditions such as previous abdominal surgery or obesity affect the detection rates of fetal anomalies (22). The detection rate will vary depending on whether the pregnant population was in low or high-risk groups. Karim et al. (8), reported that detection rates for fetal structural anomalies in low and high-risk groups were 32% and 61.18%, respectively. Liao et al. (23) reported that 43% of 1578 diagnosed fetal anomaly cases were detected in the first-trimester exam, 95.6% of abdominal anterior wall defects, 21% of urogenital anomalies, 18.4% of thoracic anomalies, and 37% of cardiac anomalies were diagnosed by routine first-trimester ultrasonography. The diagnosis rate is lower, particularly for thoracic and central nervous system anomalies (24). In this large-scale series, the diagnosis rate of gastrointestinal tract anomalies was only 4.1%.

The detection rate may be as high as 61% for pregnancies with high NT, advanced maternal age, and a previous pregnancy history of multiple fetal anomalies (2). Our cohort consisted of both high-risk and low-risk pregnancies and 55 of 90 (60.43%) of diagnosed fetal structural anomalies were detected in the first trimester exam, a similar detection rate to the literature. Nearly half of the detected congenital anomalies were congenital heart defects. This relatively higher detection rate could be linked to the study population characteristics with many fetuses having thicker NT (over 95 percentile). Furthermore, dedicated exams by maternal and fetal medicine specialists may also result in a higher diagnosis rate for congenital heart anomalies. Notably, 27.26% of fetuses with detected anomalies had multiple congenital anomalies. The co-existence of various anomalies may contribute to the earlier diagnosis of fetal defects in the first trimester.

Syngelaki et al. (25) classified fetal structural anomalies into three different categories: consistently detectable; may be detectable; and not diagnosable in the first trimester. These authors then subdivided those who could be diagnosed into those with a diagnosis rate of more than 50% and those with a diagnosis rate of less than 50%. In the same study, encephalocele, Pentalogy of Cantrell, abdominal wall defects, congenital diaphragmatic hernia, body-stalk anomaly, congenital heart defects, open neural tube defects, fetal akinesia, and lethal skeletal anomalies were reported to have the highest association with higher NT values, with 47.9% of fetuses with congenital cardiac defects have NT values higher than the 95 percentiles. In this study, 62.5% of fetuses with heart defects had increased NT (>95th percentile). Grande et al. (20) reported that all acrania, alobar holoprosencephaly, omphalocele, hydrops, megacystis, and hypoplastic left heart cases were diagnosed in the first trimester scan.

Table 5. Late diagnosed fetal anomalies following the normal first-trimester exam

Case	Weeks of gestation	Ultrasound findings	Genetic diagnosis	Prognosis
1	24 weeks	Posterior urethral valve - bilateral vesicoureteral reflux	Normal karyotype	Live delivery at 35 weeks of gestation
2	18 weeks	Bilateral pes equinovarus	-----	Term live delivery
3	21 weeks	D type transposition of great arteries	Regretted	Term delivery, postnatal exitus
4	23 weeks	Intrauterine growth restriction and lissencephaly	Triploidy	Termination of pregnancy
5	21 weeks	VSD, unilateral hypoplasia of nasal bone	Trisomy 21	Termination of pregnancy
6	21 weeks	Pelvic kidney (left)	cf-DNA test was low-risk	Term live delivery
7	22 weeks	Membranous VSD	------	Term live delivery
8	21 weeks	Sacral meningocele (16x12 mm)	------	Term live delivery
9	19 weeks	CCAM* (Right lung)	------	Term live delivery
10	20 weeks	Muscular VSD + ARSA	cf-DNA test was low-risk	Term live delivery
11	20 weeks	Unilateral renal agenesis	------	Term live delivery
13	20 weeks	Outlet VSD	Invasive test regretted	Term live delivery
14	22 weeks	Muscular VSD	invasive test regretted	Term live delivery
15	19 weeks	Outlet VSD	cf-DNA test was high-risk, invasive test regretted	Lost-follow-up
16	22 weeks	Pericardial effusion	Parvovirus Infection ? Invasive test regretted.	Lost-follow-up
17	19 weeks	Coarctation of aorta	DiGeorge	Preterm delivery
18	20 weeks	Hypoplastic left heart	Invasive test regretted	Term live delivery
19	23 weeks	Hypoplastic left heart	Invasive test regretted	Term live delivery
20	20 weeks	Unilateral nasal hypoplasia	Invasive test regretted	Term live delivery
21	22 weeks	Muscular VSD	cf-DNA test was low-risk, invasive test regretted	Term live delivery
22	20 weeks	Muscular VSD in co-twin	Invasive test regretted	Term live delivery
23	22 weeks	Absence of gallbladder	Invasive test regretted	Term live delivery
24	20 weeks	Umbilical vein varice	cf-DNA test was low-risk	Term live delivery
25	32 weeks	Fetal ovarian cyst (unilateral)	cf-DNA test was low-risk	Term live delivery
26	30 weeks	Uniloculated simple cyst (3 cm) on the upper pole of left kidney	cf-DNA test was low-risk	Term live delivery
27	35 weeks	Fetal arrhythmia (trigeminy)	cf-DNA test was low-risk	Late preterm delivery (PPROM)
28	28 weeks	Coarctation of aorta in co-twin pair	------	Preterm live delivery
29	30 weeks	Unilateral renal solitary cyst (3 cm)	------	Term delivery
30	33 weeks	Fetal ovarian cyst (unilateral)	cf-DNA test was low-risk	Term delivery
31	30 weeks	Cervical lymphangioma	-----	Term live delivery
32	35 weeks	Calcification of gallbladder wall, fetal growth restriction, polymicrogyria	Invasive test regretted	Term live delivery
33	29 weeks	Non-immun hydrops fetalis	Normal karyotype	In-utero exitus
34	30 weeks	Fetal ovarian cyst (unilateral, intracystic hemorrhage)	------	Term live delivery
35	29 weeks	Muscular VSD (fetal growth restriction at 35 weeks of gestation)	Normal karyotype	Late preterm delivery

*Congenital cystic adenomatoid malformation
VSD: Ventricular septal defect, ARSA: Anomalous right subclavian artery, PPROM: Preterm premature rupture of membranes

A recent meta-analysis by Karim et al. (26) reported that first-trimester ultrasound exams have a higher detection rate (>80%) for acrania, exomphalos, gastroschisis, and holoprosencephaly. Nevertheless, higher false positivity (24%) for suspected anomalies was the foremost handicap in the early anatomical examination. Data from Nationwide first-trimester anomaly scan in the Dutch national screening program revealed that first-trimester anomaly screening has 84.6% sensitivity for first-trimester major congenital anomalies and 31.6% for all anomalies. Positive predictive value was 40.9%, and 59.1% involved cases where anomalies were either not confirmed or resolved before 24 weeks gestation (27).

If the fetal evaluation is performed between 11 weeks and 11 weeks and 6 days, the total evaluation rate was 23.1%, while it was 63.8% between 12 weeks 6 days and 13 weeks 6 days. In this second period, 26.3% of the fetuses with renal, and 31.6% with cardiac evaluations were reported as “hesitant” or “inadequate” (26). Therefore, the family should be counselled about structural anomalies that can be diagnosed later, especially in the second and third trimesters, exam limitations, and ongoing progress of fetal development (28).

In the last decade, the definition of findings, such as intracranial translucency, brainstem-brainstem occipital bone distance, retronasal triangle, and maxillary gap, better visualisation of large vessel outflows, especially in the heart with colour Doppler, has increased the number of fetal anomalies that can be diagnosed in the first trimester (19). High-frequency linear and convex probes are gradually reducing the need for evaluation with transvaginal transducers. The combined transvaginal and transabdominal approach provides a better detection rate (62%) for congenital defects than both the transvaginal (51%) and transabdominal techniques (34%) in isolation (2). A combined approach was only required in 1.86 % of cases in our study. Syngelaki et al. (25) reported that the combined approach rate was 2-3% of all examinations. We can explain the lower rate of the combined approach needed for examination than in previous studies by a later median gestational age of ultrasound exams (12.4 weeks) in our study. Petousis et al. (17) reported the prevalence of anomaly in fetuses with normal karyotype as 1% and the diagnosis rate as 50% in their study based on the ISUOG guideline.

Among 3,254 cases in which fetal anatomy was evaluated in the first trimester, 24 cases were diagnosed in the second trimester, although no pathological finding was detected in the first-trimester examination. Maternal obesity and lower abdominal scarring could explain the delayed diagnosis of two cases with fetal congenital defects in the second trimester. However, a triploidy case has no pathological ultrasound signs until the 21st week of gestation except for early-onset growth restriction. Manegold et al. (22) conducted a cohort study between 1998 and 2008, and they reported that 116 fetuses (40%) of 8,079 fetuses examined in the first trimester had congenital anomalies. Furthermore, 6,378 fetuses had previously unremarkable first-trimester exams from the same cohort, and 102 (35 %) additional cases of congenital defects were detected. In the last step of this study, 5,044 fetuses that were evaluated as having no congenital structural defects were re-examined in the third trimester, and 44 (0.87%) abnormal ultrasound findings were found. Ficara et al. (3) reported that 67% of all congenital anomalies were detected in the first and second-trimester exams. A systematic review that covered 87 studies and 7,057,859 fetuses revealed that the first trimester scan detected 37.5% structural and 91.3% of lethal anomalies. If the second trimester exam is added to the exam protocol, combined sensitivity can reach 83.8% (29). We detected over 60% of fetal anomalies in the first-trimester exam using extended ISUOG guidelines (30). The fact that no distinction was made between low and high-risk in our cohort and that the median CRL value was 64 mm may explain the relatively high first-trimester diagnosis rates.

Study limitations

Limitations included he heterogeneous nature of the study, retrospective design, single-center nature and unavailability of neonatal exam data. The main strengths were that two maternal-fetal medicine specialists evaluated the cases and they were followed up until delivery.

Conclusion

The time between the 11-14 weeks of pregnancy is an opportunity for screening for aneuploidies and evaluating maternal health and severe fetal structural anomalies. Evaluation of fetal anatomy as late as possible in the first trimester and early second trimester will enable the detection of more structural anomalies.

Ethics

Ethics Committee Approval: *This study was approved by the Acıbadem University Ethic Commitee (approval number: ATADEK 2021-02/01, date: 28.01.2021).*

Informed Consent: *Retrospective study.*

Footnotes

Author Contributions: *Surgical and Medical Practices: T.U.K.D., Ö.P., Concept: T.U.K.D., E.K., Design: E.K., E.A., Data Collection or Processing: E.K., E.A., Analysis or Interpretation: T.U.K.D., Ö.P., Literature Search: E.K., E.A., Writing: T.U.K.D., E.K.*

Conflict of Interest: *No conflict of interest is declared by the authors.*

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A bioinformatics approach to identify potential biomarkers of high-grade ovarian cancer

Özlem Timirci Kahraman[1], Güldal İnal Gültekin[2], Deryanaz Billur[1,3], Esin Bayralı Ülker[1,3], Murat İşbilen[4], Saliha Durmuş[5], Tunahan Çakır[5], İlhan Yaylım[1], Turgay İsbir[6]

[1]Department of Molecular Medicine, Aziz Sancar Institute of Experimental Medicine, İstanbul University, İstanbul, Türkiye
[2]Department of Physiology, İstanbul Okan University Faculty of Medicine, İstanbul, Türkiye
[3]Department of Molecular Medicine, Institute of Health Sciences, İstanbul University, İstanbul, Türkiye
[4]Department of Biostatistics and Bioinformatics, Acıbadem University Faculty of Medicine, İstanbul, Türkiye
[5]Department of Bioengineering, Gebze Technical University Faculty of Engineering, Kocaeli, Türkiye
[6]Department of Molecular Medicine, Yeditepe University Faculty of Medicine, İstanbul, Türkiye

Abstract

Objective: High-grade ovarian cancer (HGOC) remains a significant therapeutic challenge due to its aggressive nature and poor prognosis. The aim was to elucidate the molecular drivers of HGOC through an integrated bioinformatics analysis.

Material and Methods: The microarray datasets (GSE6008 and GSE14764) served as the training set, while an independent microarray dataset (GSE23603) was used as the validation set. These datasets included low- and high-grade ovarian tumor samples and were downloaded from the ArrayExpress database. Selection criteria included clearly classified low-grade ovarian cancer and HGOC samples, as well as platform and sample processing methods compatibility. After normalization, differentially expressed genes (DEGs) were obtained using R software. Functional enrichment analysis [including gene ontology (GO) and pathway analysis] was performed using the DAVID database. A protein-protein interaction (PPI) network was constructed by STRING to identify hub genes associated with HGOC.

Results: A total of 106 common DEGs were identified across all three datasets, including 66 up-regulated and 40 down-regulated genes. Given the study's focus on potential oncogenic drivers, subsequent analyses prioritized the 66 up-regulated genes. The DEGs were classified into three groups by GO terms (21 biological process, 10 molecular function and 12 cellular component). Kyoto Encyclopedia of Genes and Genomes pathway analysis showed enrichment in metabolic pathways, oxidative phosphorylation, drug metabolism, and cell cycle regulation. The top nine up-regulated hub genes in the PPI network were *GMPS, RFC4, YWHAZ, CHEK1, CYC1, MRPL13, MRPL15, SDHA*, and *CLPB*.

Conclusion: The identification of these hub genes and pathways may represent an important step forward in our understanding of HGOC. While down-regulated genes may also hold biological significance, their analysis was beyond the scope of this study and warrants future investigation. Further experimental validation is needed to confirm the roles of the identified genes in disease pathogenesis and their potential as biomarkers and therapeutic targets.

Keywords: Ovarian cancer, novel biomarkers, bioinformatics analysis

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Address for Correspondence: *Özlem Timirci Kahraman*
e-mail: *ozlemtk@istanbul.edu.tr* ***ORCID:*** *orcid.org/0000-0002-2641-5613*

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Introduction

Ovarian cancer remains a significant global health concern, with a high mortality rate, primarily attributed to late-stage diagnosis (1). The World Ovarian Cancer Coalition's 2022 analysis highlights this challenge, projecting a substantial increase in global ovarian cancer deaths in the coming decades (2). Early-stage ovarian cancer is associated with a favorable prognosis, with a 5-year survival rate exceeding 90%. However, most cases are diagnosed at advanced stages, which limits treatment options and reduces survival rates (3).

The key distinctions between high-grade ovarian cancer (HGOC; Grade 3, FIGO stages III-IV) and low-grade ovarian cancer (LGOC; Grade 1, FIGO stages I-II) are rooted in their disparate molecular profiles, which in turn dictate their divergent clinical behaviors. HGOC is recognized by its high degree of genomic instability, contributing to its aggressive nature, rapid disease progression, and generally poor prognosis, often observed as widespread metastatic disease at initial diagnosis. This aggressive cellular behavior is driven by characteristic genetic alterations that present considerable obstacles to effective treatment. In contrast, LGOC is defined by a slower proliferation rate and a more stable genome, reflecting fewer overall genetic changes. Although less aggressive clinically, LGOC is driven by its own unique spectrum of molecular alterations. These divergent genetic mechanisms are crucial in explaining the varied clinical progression and differential responses to therapy observed in HGOC vs. LGOC. A comprehensive understanding of these molecular specificities is essential for advancing targeted treatment approaches and enhancing patient prognosis across both disease categories (4). This biological dichotomy underscores the need for grade-specific molecular characterization.

Surgery remains the primary treatment modality for ovarian cancer, often followed by adjuvant chemotherapy for advanced-stage disease. While advances in biological therapies, immunotherapy, and radiotherapy have emerged, treatment resistance persists as a major challenge (4).

A deeper understanding of the molecular mechanisms underlying ovarian cancer progression may be beneficial for identifying potential biomarkers and therapeutic targets (5).

High-throughput microarray analysis enables comprehensive gene expression studies, revealing the molecular complexity of ovarian cancer. Comparing expression profiles between tumor grades identifies differentially expressed genes (DEGs), offering insights into tumorigenesis (6-8).

Bioinformatics analysis now plays an important role in integrating and analyzing large-scale genomic data, enabling the identification of key genes and pathways associated with ovarian cancer (9). Recent years have witnessed significant advances in multi-omics technologies, spanning genomics to metabolomics, which are revolutionizing biomarker discovery and personalized medicine (10).

Previous studies have employed a data-driven approach, using publicly available gene expression datasets, to identify prognostic signatures associated with ovarian cancer (11). To further explore the molecular basis of ovarian cancer, we analyzed three publicly available microarray datasets (GSE6008, GSE14764, and GSE23603) from the ArrayExpress database. GSE23603 was chosen as the validation set due to its balanced HGOC/LGOC ratio (38 HGOC vs. 24 LGOC) and platform consistency (GPL570). The datasets were selected based on the following criteria: (1) inclusion of both low- and HGOC samples; (2) availability of raw gene expression data; and (3) sufficient sample size for robust statistical analysis. Samples with incomplete clinical data or unclear tumor grade classification were excluded. The primary objective of this study was to employ bioinformatics analysis to identify key genes and elucidate their potential molecular mechanisms in the distinction between LGOC and HGOC. By uncovering these molecular differences, we hope to identify potential biomarkers and therapeutic targets for HGOC.

Material and Methods

This study employed a comprehensive bioinformatics workflow to identify potential biomarkers in HGOC, as illustrated in Figure 1. The methodology included the following components:

Gene expression data sources

This study analyzed three publicly available microarray datasets (GSE6008, GSE14764, and GSE23603) retrieved from the ArrayExpress database (12), comprising gene expression profiles from 110 HGOC and 72 LGOC samples. Samples were included based on histopathological grade (HGOC: high-grade serous carcinoma; LGOC: low-grade serous/borderline tumors) as defined in the original studies (13-15). Clinical annotations (e.g., treatment history, mutation status) were unavailable and thus not used for filtering. The sample distribution was as follows:

- GSE6008: 36 HGOC, 24 LGOC (13)
- GSE14764: 36 HGOC, 24 LGOC (14)
- GSE23603: 38 HGOC, 24 LGOC (15)

GSE23603 served as the validation set due to its larger sample size and balanced HGOC/LGOC representation. This study used de-identified data; thus, no additional ethical approval was required.

A summary of dataset filtering and categorization is presented in Table 1 to detail the data selection workflow.

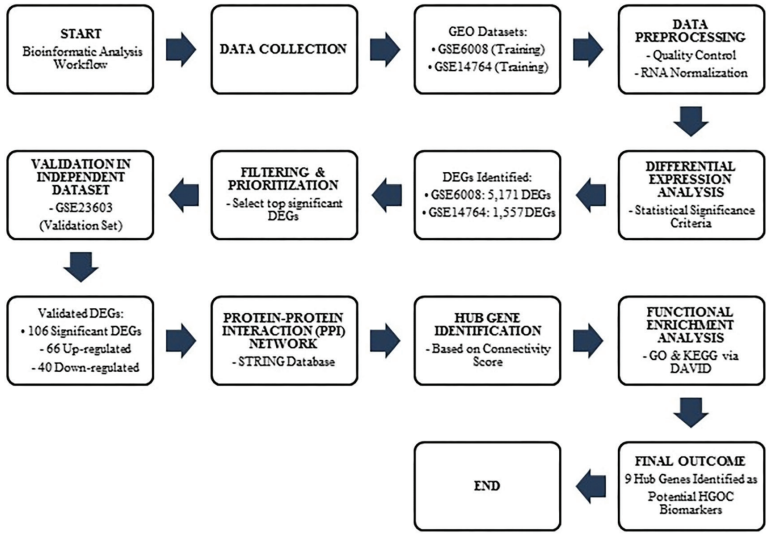

Figure 1. Flowchart of the bioinformatics workflow for identifying potential biomarkers in high-grade ovarian cancer. The diagram illustrates the step-by-step process from dataset selection through differential expression analysis, protein-protein interaction network construction, to final hub gene identification

DEGs: Differentially expressed genes, KEGG: Kyoto Encyclopedia of Genes and Genomes, GO: Gene ontology, HGOC: High-grade ovarian cancer

Table 1. Summary of dataset filtering and categorization

Dataset	Total samples	HGOC samples	LGOC samples	Platform	Study reference	Filtering criteria applied
GSE6008	60	36	24	GPL96	13	Clear histopathological grade classification, availability of raw data.
GSE14764	60	36	24	GPL96	14	Clear histopathological grade classification, availability of raw data.
GSE23603	62	38	24	GPL570	15	Clear histopathological grade classification, availability of raw data, balanced HGOC/LGOC ratio for validation.

This table summarizes the criteria and outcome of dataset selection for training and validation sets. HGOC: High-grade ovarian cancer, LGOC: Low-grade ovarian cancer

Statistical analysis

Raw data were normalized using the Robust Multi-array Average method. DEGs between HGOC and LGOC were identified using R 3.4.0 [two-tailed t-test, $p<0.05$, log2 fold change (FC) >0]. Genes were classified as up-regulated (log2 FC >0) or down-regulated (log2 FC <0); subsequent analyses prioritized up-regulated genes as potential oncogenic drivers.

Functional enrichment analysis

Gene ontology (GO) analysis categorized DEGs into molecular functions, biological processes, and cellular components. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment was performed using DAVID (16) with a significance threshold of $p<0.05$ (17), adjusted via the Benjamini-Hochberg method (18).

Candidate hub gene identification

Protein-protein interaction (PPI) networks were constructed using STRING (19,20). Hub genes were selected based on high connectivity scores and cancer pathway relevance, with the top nine up-regulated genes prioritized.

Results

A total of 5,171 DEGs were identified in GSE6008 dataset (TS1), while 1,557 DEGs were identified in GSE14764 (TS2). Comparative analysis revealed 484 overlapping genes between these datasets (Figure 2). These 484 probesets were further validated using a t-test in the independent dataset GSE23603 (VS), yielding 106 consistently dysregulated probes (66 up-regulated and 40 down-regulated genes; Table 2). Given their higher likelihood of representing oncogenic drivers in HGOC,

subsequent analyses focused on the 66 up-regulated genes.

FC values calculated across TS1, TS2, and VS demonstrated strong concordance in all pairwise comparisons (Figure 3), indicating robust experimental design and effective data normalization. The uniform distribution of FCs in scatter plots further corroborated the reliability of our differential expression analysis.

Hierarchical clustering and heatmap analysis of the 106 DEGs in the validation set (Figure 4) reinforced the stratification of samples based on gene expression profiles. For clarity, the heatmap employed a red-black-green color gradient (red: up-regulation; black: no change) and standardized labeling of samples and genes.

GO analysis categorized the DEGs into three functional groups: biological processes (21 terms), molecular functions (10 terms), and cellular components (12 terms) (Table 3).

- Biological processes were enriched for terms related to oxidative stress response (GO:0006975), regulation of cell death (GO:0043066, GO:0043065), and mitochondrial function (GO:0006121, GO:0070125), suggesting their potential disruption in HGOC progression.
- Molecular functions predominantly involved protein binding (GO:0005515) and catalytic activities linked to energy metabolism, including ATP binding (GO:0005524) and succinate dehydrogenase activity (GO:0000104), implicating altered PPIs and metabolic pathways in HGOC pathogenesis.

Cellular components highlighted enrichment in mitochondrial (GO:0005739), cytosolic (GO:0005829), and extracellular exosome compartments (GO:0070062), indicating diverse subcellular localization and potential roles in metastasis.

KEGG pathway analysis highlighted enrichment in metabolic pathways (hsa01100), oxidative phosphorylation (hsa00190), and drug metabolism (hsa00983), underscoring aberrant energy metabolism and therapeutic resistance in HGOC (Table 4). In addition, pathways associated with the cell cycle (hsa04110) and neurodegenerative diseases [e.g., Huntington's disease (hsa05016), Parkinson's disease (hsa05012)] were enriched, reflecting the complex interplay of biological processes in HGOC.

Among the 66 up-regulated genes, nine hub genes (*GMPS*, *RFC4*, *YWHAZ*, *CHEK1*, *CYC1*, *MRPL13*, *MRPL15*, *SDHA*, and *CLPB*) emerged as central nodes in the PPI network (Figure 5). This network topology underscores their potential as coordinators of the aggressive phenotype of HGOC. Pathway enrichment further emphasized their roles in metabolism, oxidative phosphorylation, and cell cycle regulation, suggesting actionable therapeutic targets.

Table 2. Screening DEGs in high-grade ovarian cancer by integrated bioinformatics analysis of microarray datasets

DEGs	Gene names
Up-regulated	*ADRM1, ARMC8, ASNS, ATAD2, ATP5F1C, ATP6V1C1, AUNIP, C8orf33, CBFA2T2, CHEK1, CLPB, COX6C, CXorf56, CYC1, DENND1A, DERL1, DPY19L4, FAM49A, FAM49B, GEMIN2, GID8, GMPS, GPRC5B, GPT, GYG, HLTF, HSF1, HSPA9, IGLC1, IGLV144, KCNH2, KIFC1, MIR80712, MRPL1, MRPL15, MRPS22, MRS2, MTERF3, MYNN, NUP155, PDCD10, PITPNM3, POLR2K, PTK2, PTP4A3, RAB7A, RAD21, RFC4, RNF114, SCRIB, SDHA, SDHAP2, SDHD, SELENOT, SPDL1, TBC1D31, TFDP2, TMX2, TUBA1B, UCKL, YWHAZ, ZBTB10, ZNF239, ZNF250, ZNF34, ZNF7*
Down-regulated	*ABHD2, ACOX3, AKAP13, ALDH3A2, BCL2, CNN2, DLC1, EEF2, ENTPD4, FUT6, GLG1HUWE1, IL20RA, KCNK1, KDM4B, KIAA1324, MAP2K6, NAT1, NLGN4X, PAPSS1, PARVA, PDLIM5, PLGLB1, PLPP2, SCNN1B, SEC14L1, SLC1A1, SLC26A2, SNORD68, SOX4, SYNJ2, TCF25, TMCO6, TMEM260, TP53I3, TPT1,TRA2A,TRADD,UGCG,VRK3*
DEGs: Differentially expressed genes	

Figure 2. Venn diagram of 484 commonly changed DEGs from the two profile datasets [GSE6008 (TS1), GSE14764 (TS2)] between low and high-grade ovarian tumor samples. Different color areas represented different datasets. The cross areas indicate the most commonly changed DEGs
DEGs: Differentially expressed genes

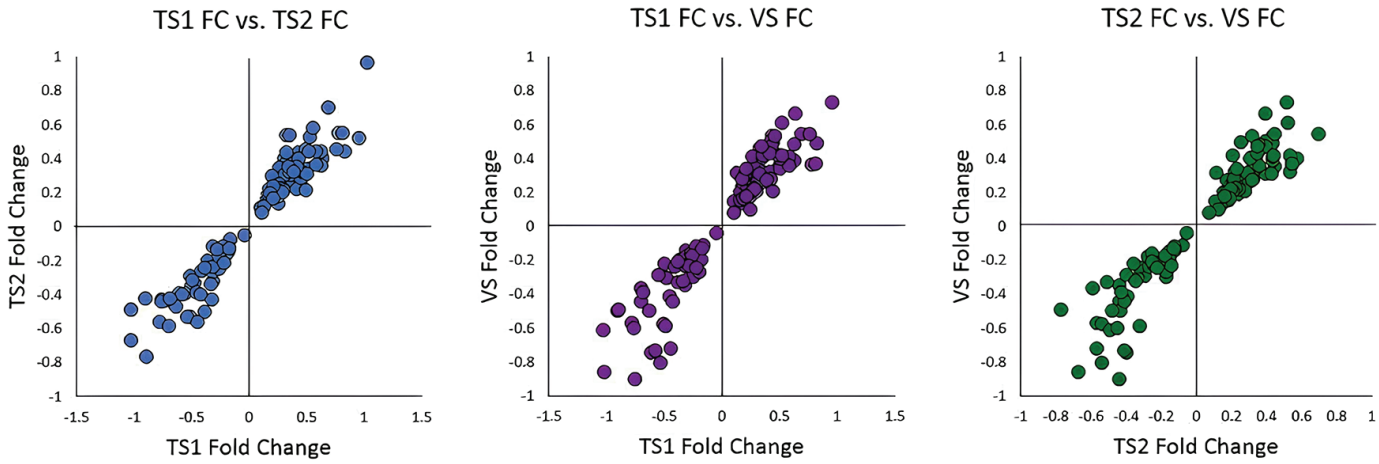

Figure 3. Comparison of "fold" change values for DEGs across the training and validation datasets
TS1: Training set 1, TS2: Training set 2, VS: Validation set, FC: fold change, DEGs: Differentially expressed genes

Table 3. GO enrichment analysis of DEGs in high-grade ovarian cancer

Category	Term code	Term	Count	p-value	Benjamini
GOTERM_BP_DIRECT	GO:0002931	response to ischemia	3	1,40E-02	1,00E+00
GOTERM_BP_DIRECT	GO:0030336	negative regulation of cell migration	4	1,50E-02	1,00E+00
GOTERM_BP_DIRECT	GO:0021747	cochlear nucleus development	2	1,60E-02	9,80E-01
GOTERM_BP_DIRECT	GO:0090168	Golgi reassembly	2	2,20E-02	9,80E-01
GOTERM_BP_DIRECT	GO:0006121	mitochondrial electron transport, succinate to ubiquinone	2	2,20E-02	9,80E-01
GOTERM_BP_DIRECT	GO:0005913	response to hydrogen peroxide	3	3,20E-02	9,90E-01
GOTERM_BP_DIRECT	GO:0050428	3'-phosphoadenosine 5'-phosphosulfate biosynthetic process	2	3,20E-02	9,80E-01
GOTERM_BP_DIRECT	GO:0051683	establishment of Golgi localization	2	3,80E-02	9,80E-01
GOTERM_BP_DIRECT	GO:0014009	glial cell proliferation	2	3,80E-02	9,80E-01
GOTERM_BP_DIRECT	GO:0043066	negative regulation of apoptotic process	7	3,90E-02	9,70E-01
GOTERM_BP_DIRECT	GO:0008360	regulation of cell shape	4	4,20E-02	9,70E-01
GOTERM_BP_DIRECT	GO:0006975	DNA damage induced protein phosphorylation	2	4,30E-02	9,60E-01
GOTERM_BP_DIRECT	GO:0035265	organ growth	2	5,30E-02	9,70E-01
GOTERM_BP_DIRECT	GO:0035023	regulation of Rho protein signal transduction	3	7,30E-02	9,90E-01
GOTERM_BP_DIRECT	GO:0070125	mitochondrial translational elongation	3	7,90E-02	9,90E-01
GOTERM_BP_DIRECT	GO:0007507	heart development	4	7,90E-02	9,80E-01
GOTERM_BP_DIRECT	GO:0000209	protein polyubiquitination	4	8,00E-02	9,80E-01
GOTERM_BP_DIRECT	GO:0070125	mitochondrial translational termination	3	8,10E-02	9,80E-01
GOTERM_BP_DIRECT	GO:0043065	positive regulation of apoptotic process	5	8,30E-02	9,70E-01
GOTERM_BP_DIRECT	GO:0048488	synaptic vesicle endocytosis	2	8,40E-02	9,70E-01
GOTERM_BP_DIRECT	GO:21747	negative regulation of anoikis	2	8,90E-02	9,70E-01
GOTERM_CC_DIRECT	GO:0016020	membrane	24	1,20E-03	1,90E-01
GOTERM_CC_DIRECT	GO:0005743	mitochondrial inner membrane	9	2,60E-03	2,10E-01
GOTERM_CC_DIRECT	GO:0005829	cytosol	30	4,00E-03	2,10E-01
GOTERM_CC_DIRECT	GO:0070062	extracellular exosome	25	1,30E-02	4,30E-01
GOTERM_CC_DIRECT	GO:0005749	mitochondrial respiratory chain complex II, succinate dehydrogenase complex (ubiquinone)	2	2,10E-02	5,30E-01

Table 3. Continued

Category	Term code	Term	Count	p-value	Benjamini
GOTERM_CC_DIRECT	GO:0005739	mitochondrion	14	2,60E-02	5,40E-01
GOTERM_CC_DIRECT	GO:0005913	cell-cell adherens junction	6	3,00E-02	5,40E-01
GOTERM_CC_DIRECT	GO:0043209	myelin sheath	4	4,90E-02	6,70E-01
GOTERM_CC_DIRECT	GO:0005856	cytoskeleton	6	5,00E-02	6,30E-01
GOTERM_CC_DIRECT	GO:0005925	focal adhesion	6	6,00E-02	6,60E-01
GOTERM_CC_DIRECT	GO:0005634	nucleus	37	8,10E-02	7,40E-01
GOTERM_CC_DIRECT	GO:0045121	membrane raft	4	1,00E-01	7,90E-01
GOTERM_MF_DIRECT	GO:0031625	ubiquitin protein ligase binding	7	5,40E-03	7,30E-01
GOTERM_MF_DIRECT	GO:0008177	succinate dehydrogenase (ubiquinone) activity	2	1,70E-02	8,70E-01
GOTERM_MF_DIRECT	GO:0019901	protein kinase binding	7	1,90E-02	7,80E-01
GOTERM_MF_DIRECT	GO:0000104	succinate dehydrogenase activity	2	2,20E-02	7,40E-01
GOTERM_MF_DIRECT	GO:0098641	cadherin binding involved in cell-cell adhesion	6	2,30E-02	6,80E-01
GOTERM_MF_DIRECT	GO:0005524	ATP binding	14	7,10E-02	9,50E-01
GOTERM_MF_DIRECT	GO:0005272	sodium channel activity	2	8,00E-02	9,40E-01
GOTERM_MF_DIRECT	GO:0016887	ATPase activity	4	8,20E-02	9,30E-01
GOTERM_MF_DIRECT	GO:0005515	protein binding	57	8,60E-02	9,10E-01
GOTERM_MF_DIRECT	GO:0009055	electron carrier activity	3	9,00E-02	9,00E-01

BP: Biological process, CC: Cellular component, DEG: Differentially expressed gene, GO: Gene ontology, MF: Molecular function

Table 4. KEGG pathway analysis of DEGs associated with high-grade ovarian cancer

Database	Pathway	ID	Gene count	p-value	Benjamini	Genes
KEGG pathway	Metabolic pathways	hsa01100	18	1,9E-3	2,2E-1	*PAPPS1, ATP6V1C1, NAT1, POLR2K, UGC, ACOX3, ALDH3A2, ASNS, COX6C, CYC1, FUT6, GPT, GMPS, PLPP2, SDHA, SDHD, SYNJ2, UCKL1*
KEGG pathway	Oxidative phosphorylation	hsa00190	5	1,3E-2	5,5E-1	*ATP6V1C1, COX6C, CYC1, SDHA, SDHD*
KEGG pathway	Drug metabolism-other enyzmes	hsa00983	3	3,9E-2	8,1E-1	*NAT1, GMPS, UCKL1*
KEGG pathway	Cell cycle	hsa04110	4	5,2E-2	6,8E-1	*RAD21, CHEK1, TFDP2, YWHAZ*
KEGG pathway	Huntington's disease	hsa05016	5	4,1E-2	7,4E-1	*POLR2K, COX6C, CYC1, SDHA, SDHD*
KEGG pathway	Amyotrophic lateral sclerosis	hsa05014	3	4,5E-2	6,9E-1	*BCL2, DERL1, MAP2K6*
KEGG pathway	Parkinson's disease	hsa05012	4	7,2E-2	7,4E-1	*COX6C, CYC1, SDHA, SDHD*
KEGG pathway	Non-alcoholic fatty liver disease	hsa04932	4	8,4E-2	7,5E-1	*COX6C, CYC1, SDHA, SDHD*

DEGs: Differentially expressed genes, KEGG: Kyoto Encyclopedia of Genes and Genomes

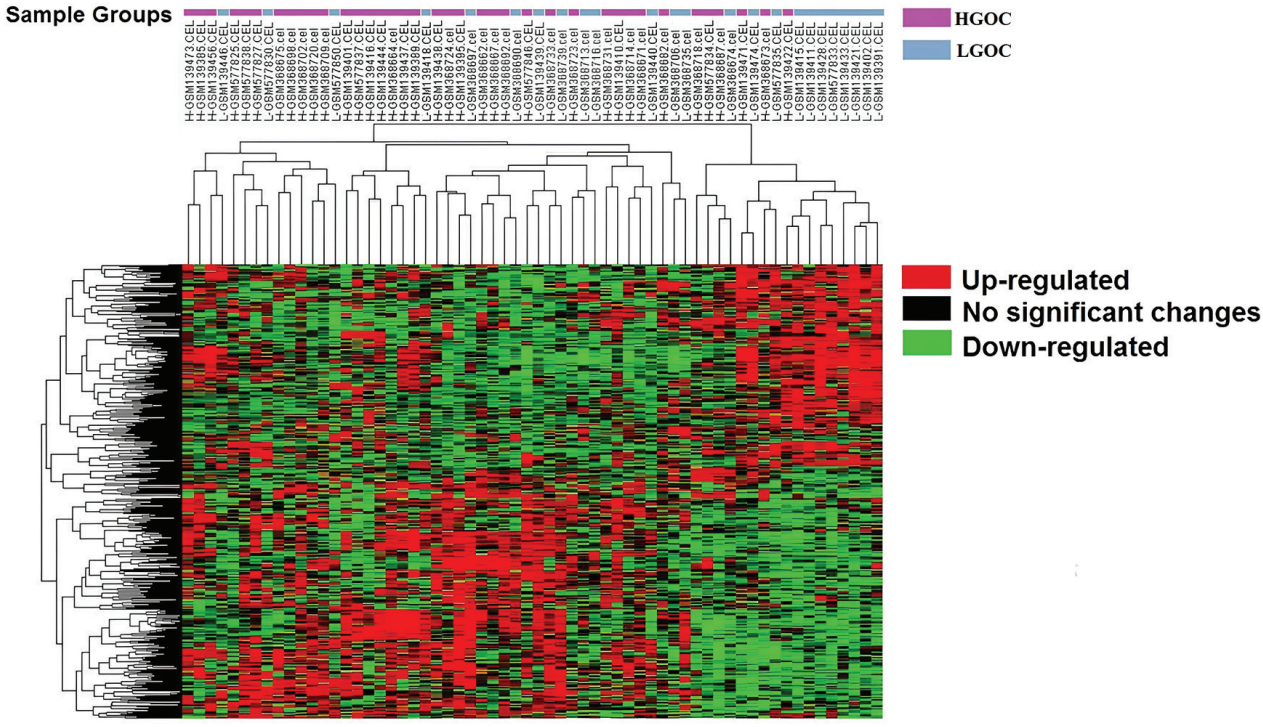

Figure 4. Hierarchical clustering heatmap of 106 DEGs. Red indicates that the expression of genes is relatively up-regulated, green indicates that the expression of genes is relatively down-regulated, and black indicates no significant changes in gene expression

DEGs: Differentially expressed genes, HGOC: High-grade ovarian cancer, LGOC: Low-grade ovarian cancer

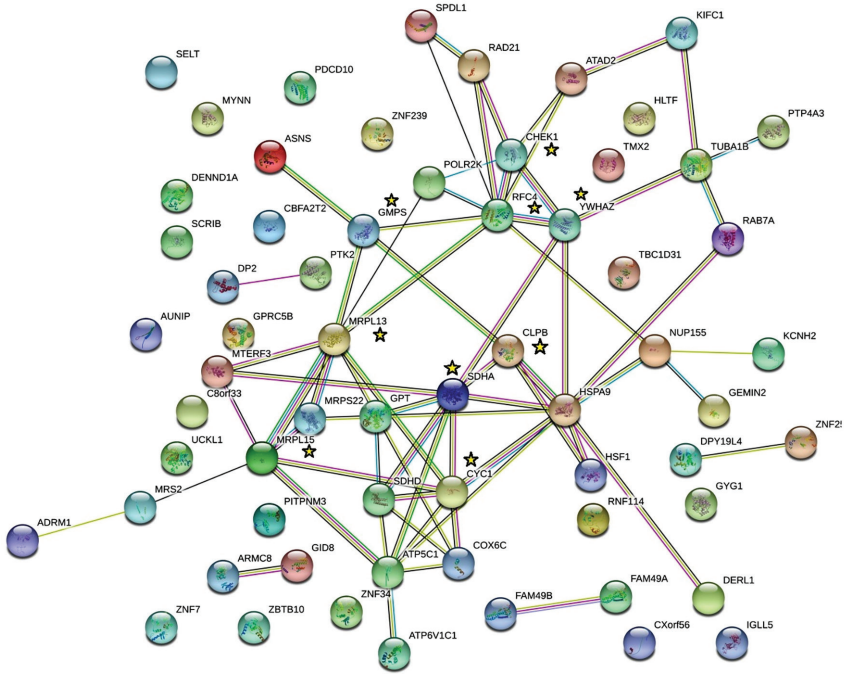

Figure 5. PPI network for up-regulated DEGs. Circles represent genes, lines represent the interaction of proteins between genes, and the results within the circle represent the structure of proteins. Line color represents evidence of the interaction between the proteins. The top nine up-regulated hub genes showing the highest expression are indicated by yellow stars

DEGs: Differentially expressed genes, PPI: Protein-protein interaction

Discussion

Ovarian cancer remains a major health challenge, with high mortality rates attributable to late diagnosis (21). While our study employs established bioinformatics methodologies, its novel contribution lies in the identification of underinvestigated genes, such as *GMPS* and *CLPB*, as possible pivotal players in HGOC pathogenesis. This finding extends beyond conventional biomarker discovery by highlighting potential therapeutic targets overlooked in prior studies. Through integrated analysis of multiple datasets, we identified 66 consistently up-regulated DEGs, predominantly enriched in metabolic and cell cycle pathways, which collectively illuminate the molecular drivers of HGOC progression.

Our decision to prioritize up-regulated genes (*GMPS*, *RFC4*, *YWHAZ*, *CHEK1*, *CYC1*, *MRPL13*, *MRPL15*, *SDHA*, and *CLPB*) was guided by three key considerations: 1) oncogenic drivers in aggressive cancers typically exhibit increased expression; 2) up-regulated genes generally engage in more extensive PPI networks than down-regulated counterparts (22); and 3) therapeutic targeting of overexpressed genes is clinically more feasible. Importantly, this strategic focus does not negate the potential relevance of down-regulated genes but aligns with translational priorities for diagnostic and therapeutic development.

Among the up-regulated hub genes identified, *GMPS*, *RFC4*, and *CLPB* emerged as particularly noteworthy due to their high connectivity in PPI networks and their understudied roles in HGOC. For instance, *GMPS* up-regulation has been linked to therapy resistance and tumor aggressiveness in other cancers (23,24), suggesting a parallel mechanism in HGOC that warrants further validation. *CHEK1*, a critical mediator of genomic stability (25), was similarly overexpressed in our analysis, corroborating findings by Lopes et al. (25) and Fadaka et al. (26) in ovarian cancer. The consistent association of *CHEK1* with tumorigenesis, particularly in DNA repair and therapy resistance, underscores its potential as a therapeutic target, as proposed in preclinical studies advocating *CHEK1* inhibition (27-30).

CLPB, though primarily recognized for its role in protein homeostasis (31), demonstrated significant up-regulation in HGOC, implying an unexplored oncogenic function. While limited studies link *CLPB* to ovarian function (32), our findings provide the first evidence of its potential involvement in HGOC pathogenesis, meriting mechanistic investigation. *YWHAZ*, a multifunctional regulator of cell proliferation and apoptosis (33,34), was similarly overexpressed, consistent with prior observations of its role in chemotherapy resistance (35) and microRNA-mediated oncogenic pathways (36). Our data reinforce *YWHAZ* as a candidate for targeted therapy, though its precise molecular mechanisms in HGOC remain to be elucidated.

Metabolic reprogramming emerged as a hallmark of HGOC in our study, exemplified by the up-regulation of *CYC1*, *MRPL13*, *MRPL15*, and *SDHA*. *CYC1*, a key component of mitochondrial respiration (37), was elevated in aggressive tumors, aligning with reports of metabolic heterogeneity in ovarian cancer (38,39) and its association with uncontrolled proliferation (40). Similarly, *MRPL13* and *MRPL15*, coding mitochondrial ribosomal proteins, were overexpressed, mirroring their documented roles in breast and lung cancers (41,42). Their association with advanced disease stages (43) and modulation of PI3K/AKT/mTOR signaling suggests a broader role in HGOC progression, possibly through mitochondrial dysfunction. *SDHA*, a critical enzyme in oxidative phosphorylation (44), further underscores the metabolic adaptability of HGOC cells, with silencing of *SDHA* shown to suppress tumor growth (44-46).

Finally, *RFC4*, a DNA replication and repair factor, was significantly up-regulated, consistent with its established association with poor prognosis (47,48). Our results not only validate *RFC4* as a prognostic biomarker but also highlight its broader role in HGOC genomic instability, offering a rationale for targeting DNA replication machinery in therapy.

While this study primarily focused on up-regulated genes as potential oncogenic drivers, it is crucial to acknowledge the biological significance of down-regulated genes, which can function as tumor suppressors or indicators of impaired cellular processes. For instance, among the 40 down-regulated genes identified, examples such as *BCL2*, *DLC1*, and *SOX4* were notable. *BCL2* is a well-known anti-apoptotic gene, and its down-regulation could indicate altered apoptotic pathways that paradoxically might contribute to drug resistance or selective survival mechanisms in certain contexts of HGOC (49).

DLC1, a Rho GTPase-activating protein, is frequently reported as a tumor suppressor in various cancers, and its reduced expression can lead to increased cell migration and invasion (50).

Similarly, SOX4 is a transcription factor with context-dependent roles, often acting as an oncogene but also demonstrating tumor-suppressive functions in some cancers (51); its down-regulation here warrants further investigation into its precise role in HGOC.

While these genes were not the primary focus, their inclusion in the identified DEGs underscores the complex molecular landscape of HGOC and opens avenues for future research into their specific contributions to disease progression and their potential as therapeutic targets.

Study limitations

This study has several inherent limitations. Firstly, our analysis primarily focuses on gene expression data, which provides a snapshot of gene activity but may not fully capture the intricate complexities of ovarian cancer. Other crucial factors, such as epigenetic modifications, PPIs, and microRNA regulation, also significantly influence tumor development and progression. Secondly, the reliance on publicly available datasets introduces potential biases arising from variations in patient populations, sample collection methods, and data processing techniques. Finally, experimental validation is crucial to confirm the functional roles of the identified genes and to explore their therapeutic potential in preclinical and clinical settings.

Conclusion

Our study identified a set of up-regulated genes, including *GMPS*, *CHEK1*, *CLPB*, *YWHAZ*, *CYC1*, *MRPL13*, *MRPL15*, *SDHA*, and *RFC4*, as potential biomarkers for HGOC. While the role of GMPS in ovarian cancer has been relatively understudied, our findings suggest its potential as a valuable biomarker. While down-regulated genes may also hold biological significance, their analysis falls outside the scope of this study, which focused on oncogenic drivers, and should be explored in future research. To fully exploit the clinical potential of these genes, a systems biology approach is necessary to understand their complex interactions within cellular networks. Further wet lab validation is crucial to translate these findings into clinical applications.

Ethics

Ethics Committee Approval: *Not applicable.*

Informed Consent: *Not applicable.*

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Footnotes

Author Contributions: *Surgical and Medical Practices: Ö.T.K., Concept: Ö.T.K., Design: Ö.T.K., T.İ., Data Collection or Processing: Ö.T.K., G.İ.G., M.İ., S.D., T.Ç., İ.Y., Analysis or Interpretation: Ö.T.K., G.İ.G., D.B., E.B.Ü., M.İ., S.D., T.Ç., İ.Y., Literature Search: Ö.T.K., D.B., E.B.Ü.,Writing: Ö.T.K., D.B.*

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Comparative analysis of hormonal and metabolic indices in phenotypic subgroups of polycystic ovary syndrome

Belgin Savran Üçok[1], Türkan Dikici Aktaş[1], Emel Özalp[1], Can Ozan Ulusoy[2], Özgür Volkan Akbulut[2], Aziz Kından[2], Fahri Burçin Fıratlıgil[3]

[1]Clinic of Obstetrics and Gynecology, University of Health Sciences Türkiye, Ankara Etlik City Hospital, Ankara, Türkiye
[2]Clinic of Perinatology, University of Health Sciences Türkiye, Ankara Etlik City Hospital, Ankara, Türkiye
[3]Clinic of Perinatology, Ankara Bilkent City Hospital, Ankara, Türkiye

Abstract

Objective: To compare hormonal and metabolic characteristics across Rotterdam polycystic ovary syndrome (PCOS) phenotypes (A–D) and identify key predictors of hyperandrogenism.

Material and Methods: In this retrospective cohort study, women with PCOS were classified into four Rotterdam phenotypes. Hormonal and metabolic parameters were assessed in the early follicular phase, and composite indices including HOMA-IR, QUICKI, TG/HDL, and free androgen index (FAI) were calculated. Logistic regression and receiver operating characteristic analysis were used to evaluate predictors of hirsutism.

Results: The study included 226 women, with respective phenotype subgroups of: A n=85; B n=29; C n=43; and D n=69. Phenotype A showed the most pronounced hyperandrogenic and metabolic alterations, whereas phenotype D displayed the mildest profile with lower androgen levels and hirsutism scores. Significant differences in insulin resistance and lipid-related indices were observed across phenotypes. FAI was the strongest predictor of hirsutism (area under the curve =0.861), followed by total testosterone and dehydroepiandrosterone sulfate, while sex-hormone binding globulin was inversely associated.

Conclusion: PCOS phenotypes demonstrate distinct hormonal and metabolic patterns. Phenotype A represents the most metabolically and androgenically severe subgroup, whereas phenotype D is comparatively mild. FAI emerges as the most informative marker for hirsutism, supporting a phenotype-oriented approach to clinical assessment and follow-up in PCOS.

Keywords: Polycystic ovary syndrome, Rotterdam phenotypes, insulin resistance, TyG index, free androgen index, hirsutism, LH/FSH, AMH

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Introduction

Polycystic ovary syndrome (PCOS) is a prevalent endocrine disorder in women of reproductive age, linked to heightened risks of subfertility, metabolic syndrome, type 2 diabetes, and cardiovascular disease (1,2). This condition, characterized by significant heterogeneity in reproductive, metabolic, and dermatological manifestations, is estimated to impact 5% to 15% of women globally (3-5).

The diagnosis of PCOS is based on the 2003 Rotterdam criteria, necessitating the presence of a minimum of two of the following: oligomenorrhea/anovulation; hyperandrogenemia; and polycystic ovarian appearance (6). The phenotypic

Address for Correspondence: *Belgin Savran Üçok*
e-mail: *dr.belgin@gmail.com* ***ORCID:*** *orcid.org/0000-0003-3429-3879*

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variation arising from these criteria results in substantial disparities in clinical presentation, diagnosis, and treatment approaches (7). The National Institute of Health criteria established in 1990 delineated two principal phenotypes for polycystic ovarian syndrome. phenotype A is characterized by the presence of hyperandrogenism, oligoanovulation, and polycystic ovary morphology, whereas phenotype B is defined by hyperandrogenism and oligoanovulation in the absence of polycystic ovarian morphology. These two phenotypes are frequently designated as the "classic" forms of PCOS. Subsequently, the 2003 Rotterdam criteria and the 2006 Androgen Excess and PCOS Society guidelines refined this classification by introducing phenotype C, characterized by hyperandrogenism and polycystic ovaries without ovulatory dysfunction, and phenotype D, defined by oligo-anovulation and polycystic ovaries in the absence of hyperandrogenism (8,9).

Although the etiology of PCOS is not definitively known, genetic, environmental, and epigenetic factors are thought to be involved. Hormonal imbalances, particularly increased luteinizing hormone (LH), androgen excess, insulin resistance, and decreased sex hormone binding globulin (SHBG), are the frequently observed biochemical changes in PCOS (10). Clinically, it can manifest with symptoms such as oligomenorrhea, anovulation, hyperandrogenism, hirsutism, acne, and hair loss (11).

Recent studies have investigated the relationship between biochemical markers and various symptoms, highlighting their importance for diagnosis and prognosis. Markers such as the LH/follicle stimulating hormone (FSH) ratio, testosterone/SHBG, anti-Müllerian hormone (AMH) levels, and the insulin/glucose ratio have been shown to play different roles in different phenotypes of PCOS. However, combining these parameters to create new indices and investigating their relationship with symptoms remains understudied.

This study will assess the predictive power of biochemical parameters in PCOS patients, including in terms of differences between different PCOS phenotypes, and based on the data obtained, the relationship between indices that can be used in diagnosis and management and PCOS subtypes will be evaluated. This will enable earlier diagnosis of PCOS in clinical practice and the development of personalized treatment plans specific to each symptom.

Material and Methods

This retrospective cohort study was performed at a single center's infertility clinic from April to July 2025. The study protocol was approved by the University of Health Sciences Türkiye, Ankara Etlik City Hospital Ethics Committee (approval number: AEŞH-BADEK2-2025-176, date: 10.06.2024), and all procedures adhered to the Declaration of Helsinki. The study comprised women with PCOS, diagnosed according to the 2018 ESHRE/ASRM criteria, which require the presence of at least two of the following features: ovulatory dysfunction, clinical or biochemical hyperandrogenism, and polycystic ovarian morphology on ultrasonography (12). Participants were categorized into the four phenotypes of PCOS.

Phenotypes were categorized as follows: phenotype A, Hyperandrogenism + ovulatory dysfunction + polycystic ovarian morphology; phenotype B, Hyperandrogenism + ovulatory dysfunction; phenotype C, Hyperandrogenism + polycystic ovarian morphology; phenotype D, Ovulatory dysfunction + polycystic ovarian morphology (13,14).

Inclusion criteria were women aged 18–35 years who had a pre-existing diagnosis of PCOS and who presented to the outpatient clinic with symptoms related to PCOS, such as menstrual irregularity, hyperandrogenic manifestations, or infertility.

Exclusion criteria included: pregnancy; postpartum or lactation period; the presence of major systemic or psychiatric disorders; non-PCOS endocrine diseases (including thyroid dysfunction, hyperprolactinemia, congenital adrenal hyperplasia, and Cushing syndrome); conditions requiring intensive care monitoring; and incomplete clinical or laboratory data.

Participants' demographic and clinical data were recorded, and body mass index (BMI) was calculated after standardized measurment of height and weight, using the standard formula. Hirsutism was assessed using the modified Ferriman–Gallwey score, with a value of ≥8 being considered to indicate clinical hyperandrogenism. Venous blood samples were collected after an 8–12-hour fast during the early follicular phase of menstruation (days 2–5). Laboratory analyses included fasting glucose, insulin, lipid profile, and hormone levels (total and free testosterone, estradiol, prolactin, dehydroepiandrosterone sulfate (DHEA-S), 17-hydroxyprogesterone, LH, FSH, thyroid stimulating hormone, tri-iodothyronine, thyroxine and SHBG.

The homeostatic model assessment of insulin resistance (HOMA-IR) calculated as [(Fasting insulin × Fasting glucose)/405], quantitative insulin sensitivity check index (QUICKI) calculated using the formula [1/(log insulin + log glucose)] and triglyceride-glucose (TyG) index [ln (triglyceride × glucose/2)] were used to assess insulin resistance and metabolic status. Free androgen index (FAI) was calculated using the formula (total testosterone × 100)/SHBG (9,15-17).

Statistical analysis

Statistical analyses were performed using SPSS, version 29.0 (IBM Corp., Armonk, NY, USA). Data distribution was assessed using visual inspection and the Kolmogorov–Smirnov test. Continuous variables are expressed as median (interquartile range) or mean ± standard deviation, as appropriate, while

categorical variables are presented as counts and percentages. Comparisons between the four PCOS phenotypes (A–D) were conducted using the Kruskal–Wallis test for non-normally distributed continuous variables and one-way ANOVA for normally distributed variables, with post-hoc pairwise comparisons adjusted using Bonferroni correction. Categorical variables were compared using the chi-square test or Fisher's exact test, as appropriate. Multinomial logistic regression analysis was used to identify independent hormonal and metabolic predictors of PCOS phenotypes, with phenotype D serving as the reference category. Results were reported as odds ratios (ORs) with 95% confidence intervals (CIs). A two-sided p-value <0.05 was considered statistically significant.

Results

Table 1 shows that age was similar across PCOS phenotypes, while BMI was significantly higher in phenotype A compared with phenotype D (p<0.001). Ovulatory dysfunction was present in phenotypes A, B, and D but absent in phenotype C. Hyperandrogenism was observed in phenotype A–C and not in phenotype D, and polycystic ovarian morphology differed significantly among phenotypes (all p<0.001).

Table 2 demonstrates significant hormonal and metabolic differences. Total and free testosterone were highest in phenotype A and lowest in phenotype D. Phenotype D had lower DHEA-S and higher SHBG levels (p<0.001), consistent with this being the mildest phenotype. Fasting insulin, triglycerides, and very low density lipoprotein levels were significantly elevated in phenotype A compared with other phenotypes.

Table 3 indicates that androgenic and metabolic indices differed across phenotypes. Phenotype A showed higher FAI, insulin resistance indices (HOMA-IR, TyG), and lower insulin sensitivity (QUICKI), whereas phenotype D displayed the most favorable metabolic profile (all p<0.05).

Table 4 shows that, using phenotype D as the reference, higher FAI was independently associated with phenotype (p<0.001). HOMA-IR was positively associated with phenotype A and B, QUICKI was inversely associated with phenotype A, and AMH was independently associated only with phenotype B.

As presented in Table 5, the FAI showed the strongest association with hyperandrogenism and the highest discriminative performance [OR =1.83, p<0.001; area under the curve (AUC) =0.861]. Total testosterone, SHBG, and DHEA-S also demonstrated significant predictive value, whereas metabolic indices showed limited or no discriminatory ability.

Discussion

This study compared the hormonal and metabolic profiles of PCOS subgroups in women of reproductive age, differentiated according to Rotterdam phenotypes (A–D). It was demonstrated that phenotype A was characterized by a more unfavorable metabolic profile (higher BMI, HOMA-IR, TyG, Tg/HDL; lower QUICKI) and significant hyperandrogenemia, while phenotype D exhibited the mildest phenotype, as expected, in terms of hyperandrogenemia markers including total testosterone, FAI and hirsutism. The study highlights the clinical implications of phenotypic heterogeneity and supports the individualization of screening and monitoring strategies based on Rotterdam phenotype.

Significant differences in reproductive and metabolic markers are reported between phenotypes in PCOS. In brief, it has been demonstrated in many studies that phenotype A has the

Table 1. Comparison of demographic, clinical, ultrasonographic, and laboratory characteristics among the four Rotterdam phenotypes of polycystic ovary syndrome

Variables	Phenotype A n=85	Phenotype B n=29	Phenotype C n=43	Phenotype D n=69	p-value
Demographics, median (IQR)					
Age (years)	25.0 (22.0, 27.0)	23.0 (21.0, 27.0)	26.0 (22.0, 29.0)	25.0 (23.0, 28.0)	0.298
BMI (kg/m²)	29.0 (25.0, 33.0)	25.0 (21.0, 34.0)	25.0 (23.0, 31.5)	24.0 (21.0, 27.0)	**<0.001**[a]
Clinical, ultrasonographic and laboratory findings, n (%), median (IQR)					
Ferriman-Gallwey score	12.0 (9.7, 15.0)	12.0 (11.0, 14.0)	12.0 (10.0, 13.0)	6.0 (5.0, 6.0)	**<0.001**[b]
Ovulatory dysfunction	85 (100.0)	29 (100.0)	0 (0.0)	69 (100.0)	**<0.001**
Hyperandrogenism	85 (100.0)	29 (100.0)	43 (100.0)	0 (0.0)	**<0.001**
PCOM	85 (100.0)	0 (0.0)	43 (100.0)	69 (100.0)	**<0.001**

Hyperandrogenism refers to the presence of clinical and/or biochemical hyperandrogenism as defined by the Rotterdam criteria
Variables with normal distribution were compared using one-way analysis of variance (ANOVA), while non-normally distributed variables were analyzed using the Kruskal–Wallis test. Categorical variables were compared using the chi-square test. Data are presented as median (interquartile range) or n (%), as appropriate. A p-value <0.05 was considered statistically significant
[a]Statistically significant differences were observed between phenotypes A vs. D
[b]Statistically significant differences were observed between phenotypes, D vs. A-C
BMI: Body mass index, PCOM: Polycystic ovary morphology, IQR: Interquartile range

most intense hyperandrogenemia and metabolic risks, while phenotype D has a profile characterized by predominant ovulatory dysfunction and weak hyperandrogenemia (18-22). Our findings are consistent with this general framework.

Previous studies have suggested that alterations in gonadotropin dynamics, particularly higher LH levels and an increased LH/FSH ratio, are more prominent in the classic PCOS phenotypes, especially phenotype A, and may help distinguish between phenotypic subgroups (18,20,23,24). In the present study, however, this pattern was not consistently replicated. In the multinomial logistic regression analysis, the LH/FSH ratio was not significantly associated with phenotype A or B when compared with the reference phenotype D, and a significant difference was observed only between phenotype C and D. Notably, this association was modest in magnitude, limiting its clinical interpretability. Overall, these findings suggest that the LH/FSH ratio may have a limited role in discriminating between PCOS phenotypes. Its clinical usefulness appears to be influenced by population characteristics and methodological variability, indicating that the LH/FSH ratio alone is unlikely to represent a reliable marker for phenotypic classification in PCOS in women of reproductive age.

Furthermore, we found AMH to be higher in phenotype A than in B and C, and lower in B than in D. Our findings support a recent meta analysis which found that AMH levels are associated with phenotypes in the sequence A > D > C > B (25), implying that the follicle pool and antral follicle number may differ according to phenotypes.

The high HOMA-IR and TyG and low QUICKI in phenotype A suggests that this phenotype is more prone to insulin resistance. Numerous studies have supported the association of the TyG index with IR and metabolic syndrome in PCOS, and its good diagnostic performance in distinguishing PCOS (26-28). In the present study, TyG values were significantly higher in phenotype

Table 2. Comparison of hormonal and biochemical parameters among the four Rotterdam phenotypes of polycystic ovary syndrome

Variables	Phenotype A n=85	Phenotype B n=29	Phenotype C n=43	Phenotype D n=69	p-value
Estradiol	38.9 (31.4, 50.0)	42.0 (35.0, 54.0)	36.0 (25.0, 48.4)	41.0 (33.6, 53.0)	0.147
FSH (mIU/mL)	5.4 (4.5, 6.3)	5.9 (5.1, 6.5)	5.6 (5.0, 6.1)	5.5 (4.7, 6.5)	0.460
LH (mIU/mL)	9.2 (7.0, 13.0)	8.4 (5.7, 12.0)	9.0 (6.2, 11.9)	11.0 (8.2, 16.0)	0.064
Prolactin (ng/mL)	15.0 (10.0, 21.0)	19.0 (14.0, 25.0)	19.0 (13.0, 23.0)	16.0 (12.0, 21.0)	0.096
TSH (μIU/mL)	1.9 (1.4, 2.8)	2.2 (1.4, 3.2)	2.4 (1.5, 3.0)	2.1 (1.5, 2.7)	0.500
T3 (ng/mL)	3.2 (2.9, 3.5)	3.2 (2.9, 3.5)	3.2 (2.9, 3.4)	3.2 (3.0, 3.4)	0.778
T4 (μg/dL)	1.1 (1.1, 1.2)	1.1 (1.0, 1.3)	1.2 (1.0, 1.3)	1.2 (1.1, 1.3)	0.675
Total testosterone (ng/dL)	60.9±21.1	48.7±11.8	51.4±16.2	40.6±13.3	**<0.001**[a]
Free testosterone (pg/mL)	2.5 (1.8, 3.3)	2.4 (1.5, 3.2)	2.6 (1.4, 3.4)	2.0 (1.4, 2.6)	0.022[b]
17-hydroxyprogesterone (ng/mL)	1.1 (0.9, 1.5)	1.0 (0.7, 1.3)	1.1 (0.8, 1.9)	1.0 (0.8, 1.3)	0.232
DHEA-S (μg/dL)	325±119.0	317±159.0	318±115.0	232±80.2	**<0.001**[c]
SHBG (nmol/L)	22.0 (17.3, 32.0)	33.0 (22.0, 45.0)	25.0 (19.5, 36.0)	42.0 (35.0, 49.0)	**<0.001**[c]
Fasting glucose (mg/dL)	91.0 (83.0, 96.0)	88.0 (81.0, 95.0)	86.0 (82.5, 94.0)	85.0 (81.0, 92.0)	0.119
Fasting insulin (μIU/mL)	18.0 (10.0, 28.8)	12.0 (9.0, 19.0)	11.3 (6.7, 19.6)	11.0 (7.6, 16.0)	**0.001**[d]
Total cholesterol (mg/dL)	177 (151, 199)	166 (147, 190)	175 (158, 188)	172 (148, 198)	0.757
LDL (mg/dL)	116±33.3	108±28.7	115±21.3	113±28.4	0.711
Triglycerides (mg/dL)	132 (102, 181)	100 (60, 156)	112 (71, 141)	98 (69, 156)	**0.019**[b]
HDL (mg/dL)	47.2±10.6	48.6±12.0	49.1±11.6	51.6±11.9	0.138
VLDL (mg/dL)	23.0 (18.0, 36.0)	18.0 (12.0, 24.0)	16.5 (13.3, 25.5)	18.0 (14.5, 25.5)	0.007[d]

Variables with normal distribution were compared using one-way analysis of variance (ANOVA), while non-normally distributed variables were analyzed using the Kruskal–Wallis test. Categorical variables were compared using the chi-square test. Data are presented as median (interquartile range) or n (%), as appropriate. A p-value <0.05 was considered statistically significant

[a]Statistically significant differences were observed between phenotypes, A vs. B-D, D vs. B, C

[b]Statistically significant differences were observed between phenotypes A vs. D

[c]Statistically significant differences were observed between phenotypes, D vs. A-C

[d]Statistically significant differences were observed between phenotypes, A vs. C, D

FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, TSH: Thyroid-stimulating hormone, T3: Total triiodothyronine, T4: Total thyroxine, DHEA-S: Dehydroepiandrosterone sulfate, AMH: Anti-Müllerian hormone, SHBG: Sex hormone-binding globulin, HOMA-IR: Homeostatic model assessment of insulin resistance, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, VLDL: Very low-density lipoprotein

A, supporting the concept that metabolic dysregulation is most pronounced in the classic PCOS phenotype. Given the strongest association with hirsutism was found for the FAI. Moreover, the high AUC value reaffirmed the usefulness of FAI in detecting biochemical hyperandrogenemia in the clinic. It has been reported that FAI offers significant accuracy in the diagnosis and phenotyping of PCOS (29). The inverse association of SHBG with hirsutism was also an expected finding (30) as a low SHBG increases the free androgen fraction, increasing the risk of clinical hyperandrogenemia.

Study limitations

Our study has several limitations. The relatively small overall sample size may have reduced the statistical power of some analyses, particularly for subgroup comparisons. In addition, the unequal distribution of participants across PCOS phenotypes (with smaller sample sizes in certain groups) may have further limited the power to detect modest differences between phenotypes. The retrospective design also limited the ability to establish causal relationships. Furthermore, unmeasured confounders such as lifestyle and genetic factors could not be completely excluded. Larger, prospective, multicenter studies with more balanced phenotype group sizes are needed to confirm and extend our findings.

Our findings highlight the importance of a phenotype-sensitive clinical screening and management approach: (i) early glycemic and lipid monitoring (TyG, HOMA-IR, Tg/HDL) and weight management for phenotype A; (ii) less focus on

Table 3. Comparison of hormonal and metabolic ındices among the four rotterdam phenotypes of polycystic ovary syndrome

Variables	Phenotype A n=85	Phenotype B n=29	Phenotype C n=43	Phenotype D n=69	p-value
FAI	8.80 (5.70, 13.00)	5.80 (2.80, 8.30)	6.50 (4.25, 10.50)	3.40 (2.10, 4.00)	**<0.001**[a]
LH/FSH ratio	1.75 (1.38, 2.22)	1.58 (1.02, 2.25)	1.57 (1.11, 2.20)	2.22 (1.36, 2.81)	**0.021**[b]
AMH	7.20 (5.60, 9.10)	4.20 (3.20, 6.50)	5.60 (4.45, 7.75)	6.85 (5.27, 8.77)	**<0.001**[c]
HOMA-IR	4.00 (2.20, 6.80)	2.70 (1.90, 4.40)	2.60 (1.40, 4.20)	2.30 (1.60, 3.30)	**<0.001**[d]
TyG index	8.74 (8.39, 9.07)	8.44 (8.01, 8.81)	8.51 (8.02, 8.71)	8.43 (7.88, 8.77)	**0.006**[e]
QUICKI	0.312 (0.290, 0.338)	0.329 (0.314, 0.345)	0.335 (0.309, 0.362)	0.335 (0.319, 0.354)	**<0.001**[e]

Variables with normal distribution were compared using one-way analysis of variance (ANOVA), while non-normally distributed variables were analyzed using the Kruskal–Wallis test. Categorical variables were compared using the chi-square test. Data are presented as median (interquartile range) or n (%), as appropriate. A p-value <0.05 was considered statistically significant
[a]Significant differences between A vs. B,D, and D vs. B, C
[b]Significant difference between C vs. D
[c]Significant differences between A vs. B, C and B vs. D
[d]Significant difference between A vs. D
[e]Significant differences between A vs. C, D
FAI: Free androgen index, LH: Luteinizing hormone, FSH: Follicle-stimulating hormone, AMH: Anti-Müllerian hormone, Tg: Triglyceride, HDL: High-density lipoprotein cholesterol, HOMA-IR: Homeostatic model assessment of ınsulin resistance, TyG index: Triglyceride-glucose index, QUICKI: Quantitative insulin sensitivity check index

Table 4. Multinomial logistic regression analysis of hormonal and metabolic predictors across polycystic ovary syndrome phenotypes, using phenotype D as the reference category

Reference category Phenotype D	Phenotype A		Phenotype B		Phenotype C	
Variables	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
FAI	1.90 (1.56-2.31)	**<0.001**	1.64 (1.33-2.04)	**<0.001**	1.90 (1.56-2.31)	**<0.001**
LH/FSH ratio	0.82 (0.61-1.08)	0.170	0.67 (0.42-1.06)	0.092	0.62 (0.41-0.94)	**0.025**
AMH	1.00 (0.99-1.00)	0.979	0.66 (0.53-0.82)	**<0.001**	1.00 (0.99-1.00)	0.801
HOMA-IR	1.28 (1.12-1.48)	**<0.001**	1.23 (1.05-1.44)	**0.009**	1.15 (0.98-1.35)	0.078
TyG ratio	1.01 (0.76-1.36)	0.898	0.79 (0.47-1.31)	0.361	0.72 (0.45-1.15)	0.171
QUICKI	0.98 (0.97-1.00)	**<0.001**	0.98 (0.97-1.00)	0.097	0.99 (0.98-1.00)	0.483

Values are presented as odds ratios (OR) with 95% confidence intervals (CI). Phenotype D (non-hyperandrogenic PCOS) was used as the reference category in the multinomial logistic regression model. A p-value <0.05 was considered statistically significant.
FAI: Free androgen index, LH: Luteinizing hormone, FSH: Follicle-stimulating hormone, AMH: Anti-Müllerian hormone, HOMA-IR: Homeostatic model assessment of insulin resistance, TyG index: Triglyceride-glucose index, QUICKI: Quantitative insulin sensitivity check index, PCOS: Polycystic ovary syndrome

Table 5. Predictive performance of hormonal and metabolic markers for hyperandrogenism

	OR	95% CI	p-value	AUC
FAI	1.83	1.51-2.22	**<0.001**	0.861
LH/FSH ratio	0.74	0.57-0.96	**0.026**	0.617
AMH (ng/mL)	1.00	0.99-1.00	0.818	0.435
HOMA-IR	1.24	1.08-1.42	**0.001**	0.624
TyG ratio	0.91	0.69-1.20	0.509	0.475
QUICKI	0.98	0.98-0.99	0.004	0.624
T. Testosterone/DHEA-S ratio	0.76	0.03-17.14	0.866	0.490
Total testosterone	1.06	1.03-1.08	**<0.001**	0.755
Free testosterone	1.47	1.10-1.97	**0.008**	0.625
17-hydroxyprogesterone	1.10	0.75-1.59	0.616	0.545
DHEA-S	1.00	1.00-1.01	**<0.001**	0.723
SHBG	0.98	0.97-0.99	**0.008**	0.796

FAI: Free androgen index, LH: Luteinizing hormone, FSH: Follicle-stimulating hormone, AMH: Anti-Müllerian hormone, HOMA-IR: Homeostatic model assessment of insulin resistance, TyG index: Triglyceride-glucose index, QUICKI: Quantitative insulin sensitivity check index, OR: Odds ratio, CI: Confidence interval, AUC: Area under the curve, SHBG: Sex hormone-binding globulin, DHEA-S: Dehydroepiandrosterone sulfate
Significant results are shown in bold (p<0.05 was considered statistically significant)

markers of hyperandrogenemia and more on management of ovulatory dysfunction for phenotype D; and (iii) strengthening biochemical confirmation with FAI and SHBG in cases of suspected hirsutism. These recommendations will reduce the impact of phenotypic heterogeneity on clinical workload and patient education.

Conclusion

PCOS phenotypes have distinct hormonal–metabolic profiles. Phenotype A was characterized by insulin resistance and hyperandrogenemia (higher HOMA-IR, TyG, TG/HDL, lower QUICKI), whereas phenotype D was the mildest with the lowest hirsutism burden. FAI was the most informative marker for hirsutism, and TyG remained associated with phenotype A after BMI adjustment, supporting simple, low-cost metabolic risk stratification. LH/FSH is population- and method-sensitive and should be interpreted contextually. Care should be phenotype-guided, and findings warrant prospective multicenter validation and threshold refinement.

Ethics

Ethics Committee Approval: *The study protocol was approved by the University of Health Sciences Türkiye, Ankara Etlik City Hospital Ethics Committee (approval number: AEŞH-BADEK2-2025-176, date: 10.06.2024), and all procedures adhered to the Declaration of Helsinki.*

Informed Consent: *Waived due to the retrospective study design.*

Footnotes

Author Contributions: *Surgical and Medical Practices: F.B.F., Concept: A.K., Design: B.S.Ü., Data Collection or Processing: E.Ö., T.D.A., Analysis or Interpretation: C.O.U., Literature Search: Ö.V.A., Writing: B.S.Ü.*

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Conventional in vitro fertilization at the age of fourties

Mete Işıkoğlu, Ayşenur Avcı, Ayşe Kendirci Çeviren, Batu Aydınuraz

Gelecek IVF, Antalya, Türkiye

Abstract

Objective: Contrary to international guidelines, intracytoplasmic injection (ICSI) has increasingly been applied to a widening range of indications. The aim of this study is to present our experience with conventional in vitro fertilization (C-IVF) in women in their forties and to contribute to the ongoing debate on whether advanced maternal age should be considered an indication for preferring ICSI.

Material and Methods: We analyzed cases of non-male factor infertility in women aged ≥40 years. The primary outcome measures were fertilization rate, implantation rate, live birth rate, and miscarriage rate.

Results: The cohort included 204 patients with a mean age of 42.30±1.97 years, a mean antral follicle count of 4.65±2.60, body mass index of 25.80±4.54 kg/m^2 and a mean duration of infertility of 4.12±4.03 years. The mean duration of stimulation was 8.73±2.22 days, with a mean gonadotropin dose of 261.82±65.25 IU. The fertilisation rate was 74.69%. A mean of 1.77±0.60 embryos were transferred resulting in an implantation rate, clinical pregnancy rate and live birth rate of 10.44%, 18.62%, 12.25% respectively.

Conclusion: Fertilisation, implantation, live birth and miscarriage rates after C-IVF are satisfactory for women ≥40 years of age. Given its lower cost, ease of application and comparable clinical outcomes, C-IVF should be considered the preferred method of fertilisation in advanced-age patients. [J Turk Ger Gynecol Assoc. 2026; 27(1): 36-42]

Keywords: IVF, ICSI, advanced reproductive age, implantation rate, clinical pregnancy rate, live birth rate

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Introduction

Although medical practice should ideally be grounded in evidence-based principles, it is undeniable that the field of medically assisted reproduction (MAR) has advanced in a predominantly technology-driven manner. When we look back at the nearly a half-century after the report of the first successful in vitro fertilization (IVF) delivery, we can recognize a list of adjunct treatments or interventions exemplifying this reality. Among these clinical, laboratory and complementary interventions, intracytoplasmic injection (ICSI) can be defined as an add-on procedure when performed in non-male factor infertility cases (1). There has been an ongoing debate for the last three decades about this widely used procedure for non-male infertility cases. Nevertheless, ICSI is the *de facto* routine insemination technique for all etiologic subgroups of infertility in many countries.

After its first introduction as a remedy in order to overcome severe male factor infertility in 1992, the use of ICSI has steadily increased, primarily to address fertilisation concerns, contributing to nearly 65% of all MAR cycles worldwide across different regions (2). Contradictory to the recommendations of the current practice guidelines of the international societies which recommend reserving ICSI for severe male factor infertility or couples with a history of total fertilisation failure (TFF) (3), the fear of fertilisation failure often drives both embryologists and clinicians to favor ICSI. As a result, ICSI has at times been portrayed as the “state of the art” in human

Address for Correspondence: *Mete Işıkoğlu*
e-mail: *sikoglu@hotmail.com* ***ORCID:*** *orcid.org/0000-0002-6506-2451*

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reproduction (4) and has been misleadingly promoted for an ever-expanding range of indications, even in the absence of clear justification.

In one of our previous studies, we demonstrated that the lower cost, ease of application, and comparable laboratory and clinical outcomes make conventional-IVF (C-IVF) the preferred fertilisation method in non-male factor infertility cases (5). After this publication, we recently reported our data comparing ICSI and C-IVF in non-male factor patients with fewer than four oocytes. The data of this particular subgroup showed that in the presence of normal semen parameters, low oocyte number should not be considered an indication to perform ICSI (6). Following these publications, we extended our analysis to another challenging subgroup: women aged ≥40 years with non-male factor infertility. The aim of the present study was therefore to investigate if C-IVF yielded satisfactory clinical outcomes in women at the limit of the age spectrum and to contribute to the ongoing debate over whether advanced reproductive age *per se* can justify the use of ICSI.

Material and Methods

Study population

This retrospective study was conducted at a private assisted reproduction center, with protocol approval obtained from the Gelecek Center for Human Reproduction – Institutional Review Board (approval number: GTB 270622, date: 27.06.2022). After the standard work up of the couple at the initial visit to the clinic, detailed signed informed consent was routinely obtained prior to enrollment in the MAR program. Patient records from all couples undergoing MAR between January 2019 and March 2022 were thoroughly reviewed and cases of non-male factor infertility in women aged ≥40 years were analysed, irrespective of the number of collected cumulus oocyte complexes (COC). Exclusion criteria included a history of TFF in a previous MAR cycle, severe male factor infertility (defined as a total progressively motile sperm count with normal morphology <100,000), prenatal genetic testing (PGT) cycles, and couples undergoing cryopreservation of embryos for any indication. C-IVF was used as the insemination method in all cases (Figure 1). Data of one cycle for each patient was included for analysis.

Controlled ovarion stimulation

Controlled ovarion stimulation (COS), pituitary suppression, fertilisation, embryo transfer, and pregnancy assessment were performed as previously described (5). Briefly, after exclusion of any ovarian cyst and endometrial lesion with a baseline ultrasound scan within the first three days of the menstrual cycle, COS with human menopausal gonadotropin (hMG, Menopur®, Ferring, Denmark) was commenced with

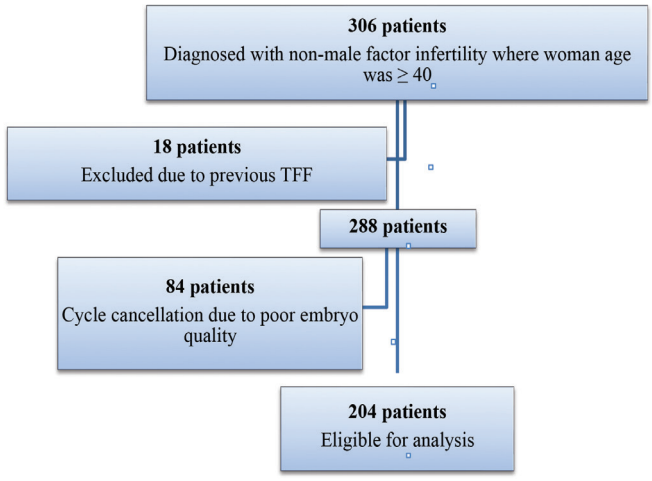

Figure 1. Flowchart showing enrollment and exclusion of the patients
TFF: Total fertilisation failure

a fixed dose of 225 IU/day, irrespective of ovarian reserve. For documentation purposes, ovarian reserve was routinely assessed on cycle day 2 or 3 of the preceding menstrual cycle using antral follicle count. Serum anti-Müllerian hormone levels were assessed in selected cases, depending on clinician preference and insurance coverage. Daily gonadotropin releasing hormone antagonist (Cetrotide® 0,25mg, Merck/Germany) injections were started using flexible protocol once the leading follicle reached 14 mm. Final oocyte maturation was triggered with 250 µg recombinant human chorionic gonadotropin (hCG, Ovitrelle®, Merck, Germany) injection when the leading follicle(s) measured ≥17 mm. Ultrasound-guided oocyte pick-up (OPU) was performed under sedation anesthesia 36 hours after hCG administration, using a single-lumen 17-gauge needle.

Fertilisation, embryo transfer and pregnancy assessment

The density gradient technique has been used as the standard method for semen preparation in our clinic. Eligibility to be enrolled in C-IVF was based on our clinical cut-off for semen quality (namely "C-IVF index" defined as total progressively motile sperm count with normal morphology ≥100,000) as previously described (6). C-IVF was used as the method of insemination in all cases achieving this threshold and ICSI was reserved only for the patients with a history of prior TFF or for couples with an index <100,000.

In the C-IVF procedure, up to three COCs were placed in each well of a four-well dish with Fertilisation Medium® (Cook, Australia) and inseminated with sperm suspension containing 100,000/mL motile spermatozoa per/COC. Maturation status of the oocytes and fertilisation, i.e. existence of two pronuclei were checked after stripping the inseminated COCs from the cumulus cells 18-20 hours after insemination.

At the cleavage stage, embryos were graded from 1 to 4 based on morphological criteria, including blastomere number, degree of fragmentation, and cell symmetry. Grade I and II embryos were deemed suitable for either transfer or cryopreservation. Single or dual cleavage-stage embryo transfers were performed on day 3 post-OPU, depending on availability.

Luteal phase support was initiated the day after OPU with daily intramuscular injections of 50 mg progesterone (Progestan 50 mg®, Koçak Farma, Türkiye). Following embryo transfer, administration was switched to the vaginal route using 200 mg progesterone capsules three times daily (Progestan 200 mg®, Koçak Farma, Türkiye) and continued until either a negative β-hCG test or the eighth week of pregnancy. The route of progesterone administration was based on convenience; using injectable preparation up to the embryo transfer ensures a clean intervention site, while switching to the vaginal form thereafter is both patient-friendly and cost-effective. The daily doses for both intramuscular and vaginal formulations were determined in accordance with the ESHRE Ovarian Stimulation Guideline.

Fertilisation rate, implantation rate, live birth rate (LBR) and miscarriage rate were the main outcome parameters.

Statistical analysis

Continuous variables are reported as mean ± standard deviation or median (quartiles), and compared between the groups with Independent Sample t-test (if the data size was sufficient in each group) or Mann-Whitney U test (if the data size was insufficient or the data was non-normally distributed in each group) based on distribution characteristics. Categorical variables are reported as numbers and percentages and compared with chi square test or derivatives, as appropriate. A two-sided $p<0.05$ was considered significant.

Results

A total of 306 cases diagnosed with non-male factor infertility where the woman's age was ≥40 years were analyzed. After excluding eighteen (5.88%) cases with a history of TFF and eighty-four cycles cancelled due to fertilisation failure or poor embryo quality, a total of 204 cases were deemed eligible for the final analysis.

Mean age of the patients was 42.30±1.97 years, mean duration of infertility was 4.12±4.03 years and mean antral follicle count was 4.65±2.60. Ninety-three (45.68%) patients had a history of previous pregnancy. Baseline characteristics of the cohort are depicted in Table 1.

Etiological reasons for infertility (Table 2) and stimulation characteristics (Table 3) are also tabulated below respectively. The mean fertilisation rate per collected COC was 74.69%. The implantation rate, LBR and miscarriage rate were 10.44%, 12.25% and 47.16% respectively (Table 4).

Table 1. Baseline characteristics of the research cohort

	C-IVF
No of patients	204
Patients' age (years)	42.30±1.97
Husbands' age (years)	42.06±6.52
Duration of infertility	4.12±4.03
Primary infertility (%)	119 (58.30%)
History of pregnancy	93 (45.68%)
Patients with previous IVF cycles	68 (33.33%)
BMI (kg/m²)	25.80±4.54
AFC	4.65±2.60

BMI: Body mass index, AFC: Antral follicle count, C-IVF: Conventional *in vitro* fertilization

Table 2. Etiological reasons for infertility

	C-IVF
Tubal factor	34
Ovulatory factor	107
Endometriosis	4
Unexplained	9
Male factor	12
Combined	38

C-IVF: Conventional *in vitro* fertilization

Table 3. Stimulation characteristics

	C-IVF
Length of stimulation	8.73±2.22
Gonadotropin units	261.82±65.25
D3 FSH	9.66±5.77
D3 LH	6.20±3.15
Peak E_2	978.47±771.44

FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, E_2: Estradiol, C-IVF: Conventional *in vitro* fertilization

Table 4. Laboratory and clinical outcome parameters

	C-IVF
COC#	960
Fertilisation rate/COC retrieved	74.69%
Fertilisation failure	18 (5.88%)
Cancelled cycles	84
#Embryos transferred	1.77±0.60
#Embryos frozen	18 (8.82%)
Implantation rate	10.44%
Pregnancy rate	53 (25.98%)
Clinical pregnancy	38 (18.62%)
Live birth rate	25 (12.25%)
Miscarriage rate	25 (47.16%)

#: Number sign, COC: Cumulus oocyte complex, C-IVF: Conventional *in vitro* fertilization

Even small time intervals can lead to a sharp decline in oocyte quality and reproductive potential in women in their forties so we further analyzed outcomes by each year of age (Table 5).

Discussion

Our results demonstrate that C-IVF provided a clinically acceptable fertilization rate, implantation rate and clinical outcome in fresh embryo transfer cycles for patients enrolling in MAR in their forties. The rationale for not including frozen embryo replacement cycles was to avoid the confounding effect of the freezing/thawing process.

Analysis of the MAR timeline shows that since the first successful delivery via C-IVF nearly fifty years ago, numerous adjunct procedures, spanning clinical, laboratory, and complementary treatments, have been introduced in efforts to improve treatment protocols and optimize fertilisation rates, embryo quality, and implantation rates. Amongst this pool of adjunct therapies and treatments, ICSI is worth being highlighted as the only prominent one so far to have been proven to contribute to the clinical outcome, albeit in male factor infertility only (1,7). Indeed, well designed randomized prospective studies and guidelines of international IVF societies have long suggested that with non-male factor infertility, C-IVF is associated with better fertilisation and implantation rates and similar LBRs when compared to ICSI (3). Hence, ICSI should actually be the insemination technique only in severe male factor infertility or reserved for patients with a history of TFF. However, during the following thirty years after the first introduction of ICSI, its use has steadily increased globally for all infertility etiologies, even accounting for 98% of all MAR cycles in certain geographical regions (8).

This increase in ICSI use may initially be attributed to some early studies claiming higher fertilisation and better clinical outcome via ICSI. Although the following well designed studies clearly showed the advantages of C-IVF over ICSI, recently factors such as patient and media-driven social pressure along with competitive dynamics within the IVF sector, have also contributed to this trend. In his article criticising liberal use of ICSI, with an analytic approach, Hans Evers ironically described IVF practitioners as Santa Claus in the fertility clinic and drew attention to the concept of therapeutic illusion, suggesting that in many cases the women who conceived with ICSI actually would also do so with C-IVF (8).

Despite the existence of robust and convincing data in favor of C-IVF, practitioners often hesitate to proceed to C-IVF in non-male factor cases for two basic reasons, both related to fertilisation. The first is the misconception that ICSI provides higher fertilisation rates, and the second and more significant concern, in our opinion, is the fear of TFF. It is therefore prudent to address these two issues separately and analyze them individually.

Fertilisation rate

When fertilisation rates are compared per inseminated oocyte, ICSI appears to yield higher rates, as expected, since immature oocytes are excluded during the denudation process. However, when assessed per collected COC, which is a more meaningful index reflecting the total number of embryos obtained, fertilisation rates are reported to be similar or even higher with C-IVF (9).

This critical point underlies the misconception that ICSI yields superior fertilisation rates (Figure 2). In fact, higher fertilisation per collected COC observed with C-IVF is quite understandable since immaturely harvested oocytes may have the chance of continuing their final maturation process in *in vitro* conditions during co-insemination with sperm suspension with their cumulus cells intact. Indeed cumulus cells are essential for supporting the growth of the oocyte, providing it with essential nutrients, hormones, and other factors crucial for proper development, maturation, fertilization, and subsequent embryonic growth.

In other words, it could be claimed that ICSI may have a detrimental effect on cycle outcomes by excluding immature oocytes from the cohort at the outset, rather than allowing them to undergo further maturation and potentially achieve fertilization in the subsequent hours. Since ICSI is an invasive procedure, another theoretical reason for better fertilisation via C-IVF is the potential degeneration of the oocytes resulting

Table 5. Clinical outcomes by maternal age increment

Age	Number of patients	+ hCG	Gestational sac	Fetal heart beat	Live birth
40	52	16 (30.8%)	14 (26.9%)	13 (25.0%)	13 (25.0%)
41	34	9 (26.5%)	4 (11.8%)	3 (8.8%)	3 (8.8%)
42	31	11 (35.5%)	8 (25.8%)	5 (16.1%)	5 (16.1%)
43	31	7 (22.6%)	4 (12.9%)	1 (3.2%)	0 (0.00%)
44	24	3 (12.5%)	2 (8.3%)	2 (8.3%)	2 (8.3%)
45	18	5 (27.8%)	2 (11.1%)	1 (5.6%)	1 (5.6%)
46	14	2 (14.3%)	2 (14.3%)	1 (7.1%)	1 (7.1%)

hCG: Human chorionic gonadotropin

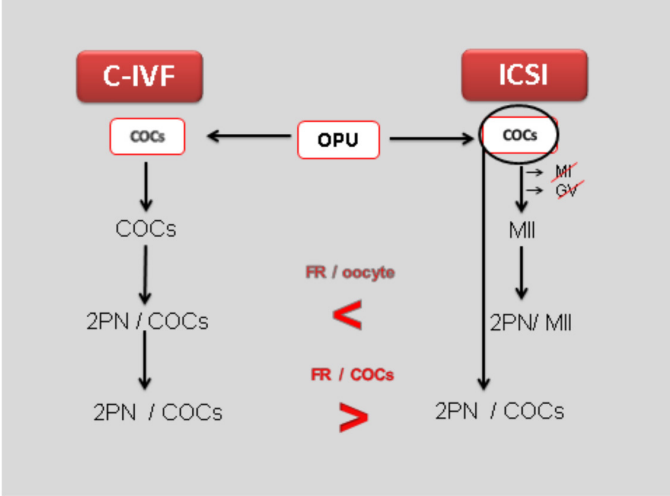

Figure 2. Fertilisation paradigm while comparing ICSI vs. C-IVF. Fertilisation rate per inseminated oocyte is better via ICSI while fertilisation rate per collected COC is better for C-IVF

COC: Cumulus oocyte complex, MI: metaphase I, MII: metaphase II, GV: Germinal vesicle, OPU: Oocyte pick-up, FR: Fertilisation rate, C-IVF: Conventional in vitro fertilization, ICSI: Intracytoplasmic injection

from mechanical damage during the denudation or ICSI procedure, which is reported to occur in 5–19% of injected oocytes. Mechanical and enzymatic removal of cumulus cells is not always simple and benign in removing all cumulus cells before ICSI, while mechanical pipetting of COCs 18-20 hours after insemination allows a faster, easier and less invasive removal of all cumulus cells.

We believe that the assumption of superior fertilisation with ICSI is more fiction than fact; the notion that "ICSI results in better fertilisation compared to C-IVF" represents a therapeutic illusion, in our opinion, in line with the suggestion of Evers. Despite the advanced age of the patients included in the present study, the fertilisation rate of nearly 75% is considered satisfactory, exceeding the 70% threshold recommended by laboratory practice guidelines.

Total fertilisation failure

Concern of encountering TFF after C-IVF is the main source of hesitancy for the practitioners preferring ICSI in cases with normal or borderline semen parameters. In fact, it is well known that ICSI does not completely exclude TFF and the literature clearly reveals similar rates of TFF with either technique (8,10). The rate of TFF observed in our advanced-age group (5.88%) was within acceptable limits and comparable to the expected TFF rates for both ICSI and C-IVF reported in international guidelines.

Beyond the misconception that ICSI yields higher fertilisation rates and the fear of TFF with C-IVF, another major reason, and perhaps the most important at present, that embryologists routinely prefer ICSI is that the young embryologists entering the field of MAR recently do not have the chance to observe or gain hands-on experience with C-IVF, resulting in limited practical knowledge of the technique. This is a growing problem which may cause the IVF team to miss a useful, time-saving, easy-to-learn and cost effective technique just because of lack of senior trainers.

Although the learning curve for C-IVF is steeper and faster than that of ICSI, there are key aspects that first-time learners must observe and practice through hands-on training, such as timing of insemination or fertilisation check, preparing the sperm suspension and calculating its volume per COC, performing rescue ICSI if the needs arise and simple manipulations at certain steps of the procedure. To support young embryologists in the field, we have prepared a freely accessible online video, "How to Do C-IVF: A Step-by-Step Guide," demonstrating each stage of the procedure in a clear and comprehensive manner (11).

Given that much of the IVF community in certain countries lacks fundamental knowledge of C-IVF, opportunities to discuss and refine the technique have been lost, including the careful selection of suitable cases, the optimal preparation of sperm suspension, and the application of alternative maneuvers (such as rescue ICSI). Focusing on the more natural and more efficient insemination technique of C-IVF should be the rationale approach instead of the fictitious belief of lower fertilisation rates or fear of TFF.

In our clinical protocol, we apply a defined metric, the "C-IVF index", both to characterize male factor infertility and to ensure the uniform use of ICSI within this group. This mathematical approach to the triage of patients for either insemination technique (ICSI or C-IVF) is helpful to the practitioners deciding the method of insemination without hesitation and also provides a standardization between practitioners. Such an index may also facilitate more meaningful comparisons between clinical and laboratory studies. To the best of our knowledge, ours is the only semen-based numerical index in the literature including all semen parameters and specifically designed to identify patients eligible for C-IVF (12).

Recently, we have published the results of a randomized controlled study comparing the laboratory and clinical outcome of ICSI with those of C-IVF in non-male factor cases (5) followed by a retrospective study comparing both techniques for patients with poor ovarian response (6) in order to clarify if lower number of oocytes would be a determinant while choosing

the insemination method. Since we found similar outcome for both C-IVF and ICSI in these two studies, we decided to further analyse our clinical data for the challenging subgroup of patiens with woman age ≥40 years to clarify if advanced age *per se* is a drawback for proceeding to C-IVF.

Demographic studies show that there is a tendency towards postponement of parenthood to late thirties and forties due to the gradual change in priorities and social life of couples in the modern era (13). Women with advanced reproductive age constitute a special group in MAR generally, with limited number of oocytes possibly with lower quality compared with the general infertility population. This raises an important question: can advanced reproductive age be considered a valid justification for preferring ICSI as the insemination method? There are a scarce number of studies in the literature directly comparing the two techniques in advanced age patients.

The first study to assess the effect of ICSI in this particular subgroup of patients was a retrospective analysis including 745 women aged 40–43 years. When C-IVF was compared with ICSI, the fertilisation rates (64% vs. 67%), fertilisation failure rates (9.0% vs. 9.7%), and LBRs (11.9% vs. 9.6%) were all comparable between the groups. Even subgroup analyses of women undergoing their first IVF cycle and women with ≤3 oocytes did not show an advantage with ICSI (13). Although this study demonstrated promising outcomes, our laboratory and clinical outcome data appear even more encouraging, considering that the patients in our cohort represent an older age population.

In another retrospective study comparing C-IVF with ICSI in 685 women aged ≥40 years with unexplained infertility, cumulative LBRs including transfers of both fresh and frozen embryo transfers were compared, with no differences observed in either cumulative live birth or abortion rates. The mean age in both groups was 41±0.8 years. Overall fertilisation rates were higher with C-IVF, while TFF tended to be more frequent in the ICSI group (6.7% vs. 9.4%) (10).

A meta-analysis evaluated the effectiveness of ICSI in improving fertilisation rates compared to C-IVF among women aged ≥38 years with a non-male factor diagnosis. A total of seven studies were included and no difference was found in fertilisation rates (14). Haas et al. (15) reported the first prospective randomized trial comparing both insemination techniques in 60 advanced age women by randomizing the ovaries of each patient prior to COS. C-IVF tended to result in a higher fertilization rate while TFF was encountered in one case from each arm (15). Taken together, all these studies support the efficiency of C-IVF with respect to both fertilization rates and subsequent clinical outcomes.

Compared with existing publications on C-IVF in advanced reproductive age, our study presents several unique findings: first, the fertilization rate per collected COC was satisfactory (74.96%); second, the rate of fertilisation failure was only slightly lower than 6%, which is acceptable given the age of the cohort; and finally, we achieved live births in women up to age 46 years, a rare and encouraging outcome. Compared to the LBR of nearly 6.5% reported by Gennarelli et al. (10), the LBR of nearly 10% of whole cohort in our study is encouraging. In our previous study, we conducted detailed comparative analyses of cost and time for both insemination techniques, demonstrating significant additional advantages of C-IVF in these respects (5). In addition to male factor infertility, there may be some relative indications for ICSI, including having a history of TFF in a previous C-IVF attempt or PGT cycles, but even these may not be strict indications.

In their well designed prospective study, De Munck et al. (16) compared the developmental competence and ploidy status in non-male factor patients undergoing PGT for aneuploidies (PGT-A) following C-IVF and ICSI on sibling oocytes and showed that ICSI is not superior to C-IVF in this regard. Hence, in cases of non-male factor infertility undergoing PGT-A, there is no strict indication to perform ICSI.

Poor oocyte quality in advanced-age women has been arbitrarily proposed as an indication for ICSI, theoretically to assist spermatozoa in bypassing some of the natural barriers. We propose the opposite analytical perspective: given that poor-quality COCs are likely more fragile, subjecting them to mechanical trauma from denudation, needle insertion, and prolonged handling outside the incubator may be detrimental. Instead, it is prudent to inseminate them via C-IVF which offers a simpler, less time-consuming approach that also avoids mechanical stress. This perspective forms the cornerstone of our strategy in managing the treatment process of infertile women of advanced age.

The present study contributes to the limited body of literature on MAR outcomes in women in their forties. By providing data on the efficacy of C-IVF from a country located in a region where ICSI is performed at an exceptionally high, and almost routine, rate (17), this study may encourage practitioners in the region to reconsider and potentially revise their standard insemination practices. Another strength of the present study is the use of a defined numerical index to characterize male factor infertility, along with the consistent application of C-IVF above a specified threshold.

Study limitations

Certain limitations, such as the retrospective design and relatively small sample size, warrant cautious interpretation of the present findings. Since C-IVF has been the default insemination method in our clinical practice for non-male factor cases for more than a decade, establishing a control group was not possible in this retrospective study. Comparison with an age-matched male factor infertility group from the same period would be inappropriate, as the adverse effects of poor sperm quality could introduce additional confounding factors.

Building on the encouraging findings of the present analysis, our next step will be to conduct a randomized study including an age-matched non-male factor infertility group undergoing ICSI as the control arm.

Conclusion

The findings of this study suggest that advanced maternal age alone should not be considered a sufficient indication for preferring ICSI over C-IVF, as C-IVF achieves satisfactory fertilization, implantation, clinical pregnancy, and miscarriage rates. Moreover, C-IVF offers advantages in terms of cost-effectiveness and time efficiency, while avoiding the potential long-term risks associated with the artificial selection of spermatozoa. Although the limited number of randomized trials makes it challenging to establish definitive conclusions regarding the superiority of either insemination method, it appears prudent to reserve ICSI primarily for cases of severe male factor infertility, in our opinion and experience.

Ethics

Ethics Committee Approval: *This retrospective study was conducted at a private assisted reproduction center, with protocol approval obtained from the Gelecek Center for Human Reproduction – Institutional Review Board (approval number: GTB 270622, date: 27.06.2022).*

Informed Consent: *After the standard work up of the couple at the initial visit to the clinic, detailed signed informed consent was routinely obtained prior to enrollment in the MAR program.*

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Footnotes

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Diagnostic and prognostic role of maternal serum prokineticin-1 in preeclampsia and adverse pregnancy outcomes

Merve Ayas Özkan[1], Aziz Kından[1], İslam Aslanlı[2], Kadriye Yakut Yücel[1]

[1]Clinic of Perinatology, University of Health Sciences Türkiye, Ankara Etlik City Hospital, Ankara, Türkiye
[2]Clinic of Obstetrics and Gynecology, University of Health Sciences Türkiye, Ankara Etlik City Hospital, Ankara, Türkiye

Abstract

Objective: Prokineticin-1 (PROK-1), known as endocrine gland-derived vascular endothelial growth factor, is an angiogenic peptide mostly produced in endocrine and placental organs. It is important for placental vascular remodeling and trophoblast control. This study sought to investigate the potential of maternal serum PROK-1 levels as a diagnostic or prognostic marker in preeclampsia (PE).

Material and Methods: This prospective case–control study included women diagnosed with PE and normotensive pregnant controls. Serum samples were collected at hospital admission, and PROK-1 concentrations were quantified using a commercial ELISA kit. Clinical characteristics and perinatal outcomes were compared between groups. Receiver operating characteristic analyses were used to assess the diagnostic and prognostic performance of PROK-1 for PE, disease severity, and composite adverse perinatal outcomes (CAPO).

Results: There were 45 women in the PE group and an equal number of controls. PROK-1 levels were significantly higher in PE than in controls [8.37 (10.51) vs. 4.89 (3.26) ng/mL, p<0.001). PROK-1 predicted PE with an area under the curve (AUC) of 0.721 (cut-off >5.40 ng/mL; sensitivity 75.6%, specificity 60.0%; positive predictive value 65.5%, negative predictive value 71.4%). Furthermore, severe PE cases had significantly higher PROK-1 levels than mild PE cases. PROK-1 predicted severe PE with an AUC of 0.716 (cut-off >9.80 ng/mL) and CAPO with an AUC of 0.673 (cut-off >6.53 ng/mL).

Conclusion: Maternal serum PROK-1 was elevated in PE and correlated with disease severity and adverse perinatal outcomes. Although inadequate as a stand-alone marker, PROK-1 may complement existing angiogenic biomarkers in multimarker prediction models. [J Turk Ger Gynecol Assoc. 2026; 27(1): 43-50]

Keywords: Preeclampsia, prokineticin-1, biomarkers, pregnancy outcome, angiogenesis

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Introduction

Preeclampsia (PE) is one of the major hypertensive disorders of pregnancy and typically occurs after the 20th week of gestation, presenting with hypertension and various systemic symptoms (1). It is a multisystemic disorder that provides considerable health hazards to both the mother and the fetus, significantly contributing to maternal and perinatal morbidity and mortality globally (2,3). The global prevalence of PE is estimated to range between 2% and 8% of all pregnancies, though regional variations are common (1). PE is clinically categorized into early-onset (EO-PE), occurring prior to 34 weeks of gestation, and late-onset (LO-PE), which presents beyond 34 weeks (4). EO-PE is often more severe and predominantly associated with fetal complications, while LO-PE tends to be more frequent and more closely linked with maternal outcomes (5). Despite these clinical distinctions,

Address for Correspondence: *Merve Ayas Özkan*
e-mail: *merveayasozkan@gmail.com* ***ORCID:*** *orcid.org/0000-0002-2437-9719*

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both forms share a common underlying mechanism involving placental dysfunction. The predominant hypothesis for the pathophysiology of PE focuses on insufficient remodeling of the spiral arteries, resulting in placental hypoperfusion, ischemia, and eventual systemic endothelial dysfunction, associated with inflammatory activation (6). This cascade of events disrupts maternal vascular homeostasis and gives rise to the clinical manifestations of PE. Molecular pathways and biomarkers have been investigated to better understand this process and to identify early diagnostic indicators (7,8).

Prokineticin-1 (PROK-1) has emerged as a notable candidate molecule. PROK-1, often referred to as endocrine gland-derived vascular endothelial growth factor (VEGF), is an angiogenic peptide predominantly found in steroidogenic tissues, including the adrenal gland, ovary, testis, and placenta (9). Although not a member of the VEGF family, it exerts VEGF-like effects in endocrine tissues by promoting vascular growth and cellular proliferation (10). PROK-1 contributes to endometrial and placental angiogenesis and regulates cellular processes including proliferation, adhesion, and invasion (11). It plays an essential role in embryo implantation and trophoblastic invasion, two of the key events in early placental development (11,12). Dysregulation of PROK-1 expression, whether excessive or deficient, has been implicated in several pregnancy complications, suggesting that it may act as a sensitive indicator of placental health (13,14).

However, despite increasing biological evidence, the clinical utility of PROK-1 in PE remains unclear. Only a limited number of human studies have evaluated circulating PROK-1 levels in PE, and findings are inconsistent, with some reporting elevated levels while others found no significant difference between PE and healthy pregnancies. Moreover, most published work has focused solely on diagnostic comparison and has not examined the relationship between PROK-1, disease severity, or adverse perinatal outcomes. Therefore, there is a knowledge gap regarding whether maternal PROK-1 levels may provide diagnostic or prognostic value in PE, beyond currently used clinical parameters.

Given these uncertainties and the lack of consistent clinical evidence, PROK-1 may be a promising biomarker for the diagnosis and prognosis of PE due to its dual role as an angiogenic and trophoblast regulatory factor. Therefore, the objective of the present study was to measure maternal serum PROK-1 concentrations in women with PE, evaluate their diagnostic and prognostic performance, and investigate their potential association with adverse perinatal outcomes.

Material and Methods

This prospective case–control observational study was conducted in the perinatology clinic of a tertiary referral center between April 2024 and October 2024, in accordance with the STROBE guidelines for observational studies. The study protocol was approved by the University of Health Sciences Türkiye, Ankara Etlik City Hospital Ethics Committee (approval number: 2024-252, date: 03.04.2024), and all procedures adhered to the Declaration of Helsinki.

During the study period, all women admitted to the perinatology clinic and evaluated for suspected PE were screened. No randomization procedures were used because this was an observational case–control design. Pregnant women aged 18–40 years who met the diagnostic criteria of the American College of Obstetricians and Gynecologists for PE were included in the case group (1). EO-PE was defined as diagnosis before 34 weeks, and LO-PE as diagnosis at or after 34 weeks of gestation (5). To minimize selection bias and ensure comparability between groups, the control group was composed of the next eligible healthy pregnant woman admitted to the clinic on the same day as each PE case, matched according to maternal age, gestational week, and body mass index (BMI). Exclusion criteria consisted of multiple current pregnancy, chronic maternal disease, fetal growth restriction diagnosed before PE, fetal congenital or chromosomal anomalies, placental pathology, chronic medication use, maternal smoking or alcohol consumption, and loss to follow-up.

The sample size was calculated using G*Power 3.0.10 with an alpha of 0.05 and a power of 0.80, indicating that a minimum of 42 participants was required. Maternal demographic characteristics (age, parity, BMI), gestational age at diagnosis, gestational age at blood sampling, gestational age at delivery, umbilical artery systolic/diastolic ratio (UA-S/D) and pulsatility index (UA-PI), biochemical parameters [aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, uric acid, 24-hour urine protein], birth weight, mode of delivery, neonatal outcomes and the presence of composite adverse perinatal outcomes (CAPO) were obtained from standardized electronic hospital records. CAPO was defined as the occurrence of at least one adverse outcome, including spontaneous preterm birth, fetal growth restriction, oligohydramnios or polyhydramnios, preterm premature rupture of membranes, fetal distress [Category III fetal heart rate tracing (15)], or the need for neonatal intensive care unit (NICU) admission.

For PROK-1 analysis, maternal venous blood samples were collected at the time of diagnosis, placed in EDTA tubes, centrifuged within 10 minutes, and stored at –80 °C until analysis. Serum PROK-1 levels were measured in duplicate using a commercially available human PROK-1 ELISA kit (E4173Hu), and results were expressed in ng/mL. The same laboratory protocol was used for all samples in both groups to ensure measurement consistency and reduce information

bias. Additional potential sources of bias were minimized by prospective participant selection, matching of controls, and the use of objective data extracted from electronic medical records.

Statistical analysis

All statistical analyses were performed using IBM SPSS, version 30.0 (IBM Inc., Armonk, NY, USA). The Kolmogorov–Smirnov test was used to assess the normality of data distribution since each group included more than 30 participants. Normally distributed variables are presented as mean ± standard deviation and compared using the Student's t-test, whereas non-normally distributed variables are presented as median (25^{th}–75^{th} percentile) and compared using the Mann–Whitney U test. Categorical variables are expressed as frequencies and percentages and analyzed using Pearson's chi-square or Fisher's exact test, as appropriate. Receiver operating characteristic (ROC) curve analysis was conducted to evaluate the diagnostic and prognostic performance of maternal serum PROK-1 levels for the prediction of PE, severe PE, and CAPO. Cut-off values were determined using the Youden index, and area under the curve (AUC) values are reported with 95% confidence intervals. No missing data were present in the study. A p-value <0.05 was considered statistically significant.

Results

A total of 90 pregnant women were included in the study, comprising 45 women with PE and 45 normotensive controls. The flowchart summarizing patient screening, exclusions, and final group distribution is presented in Figure 1. Maternal age, gravida, parity, and BMI did not differ between the groups (all p>0.05). In comparison to controls, the gestational week of diagnosis in the PE group was substantially earlier (median 33.2 weeks, p<0.001). The PE group had significantly increased UA-S/D and UA-PI (both p<0.001). Preeclamptic patients had significantly lower platelet counts (p=0.048), and their levels of AST, ALT, creatinine, and uric acid were all significantly higher than those of the control group (p<0.05 for all). The EO-PE had substantially earlier gestational weeks at diagnosis and sampling (both p<0.001) and higher UA-PI values (p=0.006) than the LO-PE when PE patients were stratified by PE subtype, whereas other maternal characteristics did not differ significantly (Table 1).

The perinatal and neonatal outcomes were markedly inferior in the PE cohort. The gestational age at delivery was significantly lower in the PE group compared to controls (36.1 vs. 39.0 weeks, p<0.001), and the mean birth weight was substantially diminished (2550 g vs. 3160 g, p<0.001). The incidences of preterm delivery (53.3% vs. 6.7%), low birth weight (<2500 g; 48.9% vs. 8.9%), and fetal growth restriction (22.2% vs. 0%) were markedly elevated in the PE group (all p<0.001). Neonates delivered by mothers with PE exhibited diminished Apgar scores at both one and five minutes (p=0.006 and p=0.003, respectively) and necessitated admission to the NICU more often (48.9% vs. 4.4%, p<0.001). CAPO were observed in 48.9% of the PE group and 6.7% of controls (p<0.001). Within PE subgroups, EO-PE was associated with higher rates of preterm delivery, low birth weight, fetal distress, NICU admission, and CAPO compared with LO-PE (p<0.05 for all) (Table 2).

The entire PE group had considerably higher median maternal serum PROK-1 levels than the controls [8.37 (10.51) vs. 4.89 (3.26), p<0.001]. PROK-1 concentrations were greater in the EO-PE and LO-PE groups than in the controls (p=0.006 and p=0.019, respectively), but there was no difference between the two PE sub-groups (p=0.467). Severe PE cases showed significantly higher PROK-1 levels than mild PE cases when analyzed by illness severity [10.75 (10.61) vs. 6.84 (6.75), p=0.014] (Table 3).

ROC analyses evaluating the diagnostic and prognostic value of PROK-1 are presented in a single composite figure (Figure 2). PROK-1 demonstrated significant diagnostic ability for PE (AUC 0.721, 95% CI 0.61–0.82, p<0.001) with an optimal cut-off value of >5.40 ng/mL (sensitivity 75.6%, specificity 60.0%). For predicting CAPO, the AUC was 0.673 (95% CI 0.55–0.79, p=0.004) with a cut-off >6.53 ng/mL. For assessing severity of PE, PROK-1 yielded an AUC of 0.716 (95% CI 0.56–0.86, p=0.006), with a cut-off of >9.80 ng/mL providing 70.0% sensitivity and 72.0% specificity. Summary performance metrics are presented in Table 4.

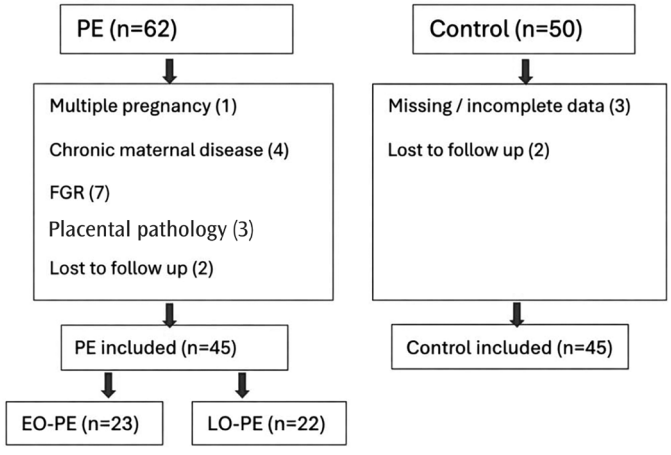

Figure 1. Flowchart of participant screening, exclusion criteria, and final group allocation. In this prospective case–control study, 62 women evaluated for preeclampsia and 50 healthy pregnant women were screened. After applying exclusion criteria, 45 women with preeclampsia and 45 normotensive controls were included in the final analysis EO-PE: Early-onset, LO-PE: Late-onset

Table 1. Maternal characteristics, Doppler findings, and biochemical markers in preeclampsia and control groups

	Total PE (n=45)	Total Control (n=45)	p-value	EO-PE (n=23)	LO-PE (n=22)	p-value
Maternal age (year)	32.1±5.8	29.5±5.2	0.062	32.4±6.0	31.9±5.6	0.781[a]
Gravida	2 (2)	2 (2)	0.320	2 (2)	2 (1)	0.267[b]
Parity	1 (2)	1 (1)	0.489	1 (2)	1 (1)	0.520[b]
Nulliparous	18 (40.0)	22 (48.9)	0.410[c]	7 (30.4)	11 (50.0)	0.173[c]
BMI (kg/m^2)	31.5 (5.8)	29.6 (5.4)	0.058[b]	31.1 (5.9)	32.8 (4.6)	0.142[b]
Gestational week at diagnosis	33.2 (5)	-	-	30.2 (5)	35.8 (2)	**<0.001**[b]
Gestation at sampling	33.2 (5)	33.5 (6)	0.318[b]	30.4 (5)	36.2 (2)	**<0.001**[b]
UA-S/D	3.0 (0.7)	2.3 (0.8)	**<0.001**[b]	3.3 (0.9)	2.6 (0.6)	**0.004**[b]
UA-PI	1.02 (0.25)	0.79 (0.21)	**<0.001**[b]	1.12 (0.27)	0.91 (0.22)	**0.006**[b]
Platelet count (10^9/L)	220 (68)	247 (74)	0.048[b]	212 (88)	229 (62)	0.521[b]
AST (IU/L)	24 (16)	16 (9)	**0.012**[b]	27 (18)	21 (13)	0.094[b]
ALT (IU/L)	19 (11)	12 (7)	**0.004**[b]	22 (13)	16 (9)	**0.046**[b]
Creatinine (mg/dL)	0.68 (0.15)	0.44 (0.09)	**<0.001**[b]	0.71 (0.16)	0.66 (0.12)	0.287[b]
Uric acid (mg/dL)	6.5 (2.2)	3.7 (1.1)	**<0.001**[b]	7.1 (2.3)	5.9 (1.8)	**0.041**[b]
24-hour urine protein (mg)	612 (1215)	-	-	748 (298)	482 (276)	0.081[b]

Data are expressed as n (%), mean ± standard deviation or median (interquartile range) where appropriate. A p-value of <0.05 indicates a significant difference and statistically significant p-values are in bold. EO-PE: Early onset preeclampsia, LO-PE: Late onset preeclampsia, BMI: Body mass index, UA-SD: Umbilical artery systolic/diastolic ratio, UA-PI: Umbilical artery pulsatility index, [a]: Student's t-test, [b]: Mann-Whitney U test, [c]: Pearson chi-square test, [d]: Fisher's exact test

Table 2. Perinatal and neonatal outcomes in preeclampsia subgroups compared to controls

	Total PE (n=45)	Total control (n=45)	p-value	EO-PE (n=23)	LO-PE (n=22)	p-value
Gestational age at delivery (week)	36.1 (3.2)	39.0 (2.1)	**<0.001**[a]	34.2 (3.9)	37.3 (2.0)	**<0.001**[a]
Preterm birth	24 (53.3%)	3 (6.7%)	**<0.001**[b]	18 (78.3%)	6 (27.3%)	**0.002**[b]
Mode of delivery Vaginal delivery Primary cesarean section Repeat cesarean section	 16 (35.5%) 21 (46.6%) 8 (17.8%)	 28 (62.2%) 10 (22.2%) 7 (15.6%)	0.005b	 6 (26.0%) 12 (52.1%) 5 (21.7%)	 10 (45.4%) 9 (40.9%) 3 (13.6%)	0.074[b]
Birth weight (gram)	2550 (1220)	3160 (580)	**<0.001**[a]	1810 (910)	2760 (780)	**<0.001**[a]
Low birth weight (<2500 gr)	22 (48.9%)	4 (8.9%)	**<0.001**[b]	17 (73.9%)	5 (22.7%)	**<0.001**[b]
FGR	10 (22.2%)	0 (0%)	**<0.001**[b]	8 (34.8%)	2 (9.1%)	**0.025**[b]
Premature rupture of membranes	4 (8.9%)	2 (4.4%)	0.403[b]	2 (8.7%)	2 (9.1%)	0.971[b]
Fetal Distress	9 (20.0%)	3 (6.7%)	0.083[b]	7 (30.4%)	2 (9.1%)	0.049[b]
Apgar score at 1st minute	8 (2)	9 (1)	**0.006**[a]	7 (3)	9 (1)	**0.002**[a]
Apgar score at 5th minute	9 (2)	10 (1)	**0.003**[a]	8 (2)	10 (1)	**0.001**[a]
RDS	12 (26.7%)	1 (2.2%)	**<0.001**[b]	9 (39.1%)	3 (13.6%)	0.064[b]
NICU admission	22 (48.9%)	2 (4.4%)	**<0.001**[b]	17 (73.9%)	5 (22.7%)	**<0.001**[a]
CAPO	22 (48.9%)	3 (6.7%)	**<0.001**[b]	17 (73.9%)	5 (22.7%)	**<0.001**[a]
Intrauterine fetal demise	1 (2.2%)	0 (0%)	0.312[b]	1 (4.3%)	0 (0%)	0.328[b]

Data are expressed as n (%), mean ± standard deviation or median (interquartile range) where appropriate. A p-value of <0.05 indicates a significant difference and statistically significant p-values are in bold. EO-PE: Early onset preeclampsia, LO-PE: Late onset preeclampsia, FGR: Fetal growth restriction, NICU: Neonatal intensive care unit, CAPO: Composite adverse perinatal outcome. [a]: Mann-Whitney U test, [b]: Pearson chi-square, [c]: Student's t-test, [d]: Fisher's exact test

Discussion

The main objective ofthe present study was to ascertain maternal serum PROK-1 concentrations in pregnancies affected by PE and to compare them with levels in healthy controls. A secondary objective was to assess the relationship between PROK-1 levels, meaures of PE severity, and pregnancy outcomes. Our findings demonstrated that maternal PROK-1 levels were significantly elevated in women with PE compared with normotensive pregnancies. The increase was most pronounced in severe PE cases and showed an additional association with CAPO. These findings suggest that PROK-1, a peptide with recognized angiogenic and trophoblastic regulatory roles, may play a clinically important role in the pathophysiology of PE. By integrating both severity and perinatal outcomes, this study has expanded the limited literature about PROK-1 and provides new insight into its clinical relevance.

During a typical pregnancy, extravillous trophoblasts infiltrate and restructure spiral arteries into low-resistance channels, facilitating optimal uteroplacental blood circulation. When this process fails, trophoblastic invasion becomes shallow and spiral artery remodeling remains incomplete, leading to placental hypoperfusion and ischemia. These events trigger systemic endothelial dysfunction and inflammation, both central to the development of PE (6,16).

PROK-1 is abundantly expressed in the placenta and functions as a key regulator of angiogenesis, vascular remodeling, and trophoblast activity (17). Physiologically, its expression peaks during the first trimester, which corresponds to the period of most intense vascular development, but then declines in later trimesters (17,18). This early elevation supports implantation and the establishment of placental circulation (17,19). PROK-1 has been shown to play an important role in the development of the normal placenta (20). Persistent elevation beyond the first trimester, however, may disrupt normal placental physiology. PROK-1 has been shown to inhibit extravillous trophoblast invasion and migration while promoting cell proliferation (17,18). These effects can lead

Table 3. Comparison of PROK-1 levels between preeclampsia subgroups and controls

Comparison Groups	Group	n	PROK-1 levels	p-value
Total-PE vs. control	**Total-PE**	45	8.37 (10.51)	**<0.001**[a]
	Control	45	4.89 (3.26)	
EO-PE vs. control	**EO-PE**	23	8.59 (8.45)	**0.006**[a]
	Control	23	4.89 (2.75)	
LO-PE vs. control	**LO-PE**	22	8.09 (6.44)	**0.019**[a]
	Control	22	4.93 (5.74)	
EO-PE vs. LO-PE	**EO-PE**	23	8.59 (8.45)	0.467[a]
	LO-PE	22	8.09 (6.44)	
Severe PE vs. Mild PE	**Severe PE**	20	10.75 (10.61)	**0.014**[a]
	Mild PE	25	6.84 (6.75)	

Data are expressed as n (%) or median (interquartile range) where appropriate. A p-value of <0.05 indicates a significant difference and statistically significant p-values are in bold. EO-PE: Early onset preeclampsia, LO-PE: Late onset preeclampsia, [a]: Mann-Whitney U Test, PROK-1: Prokineticin-1

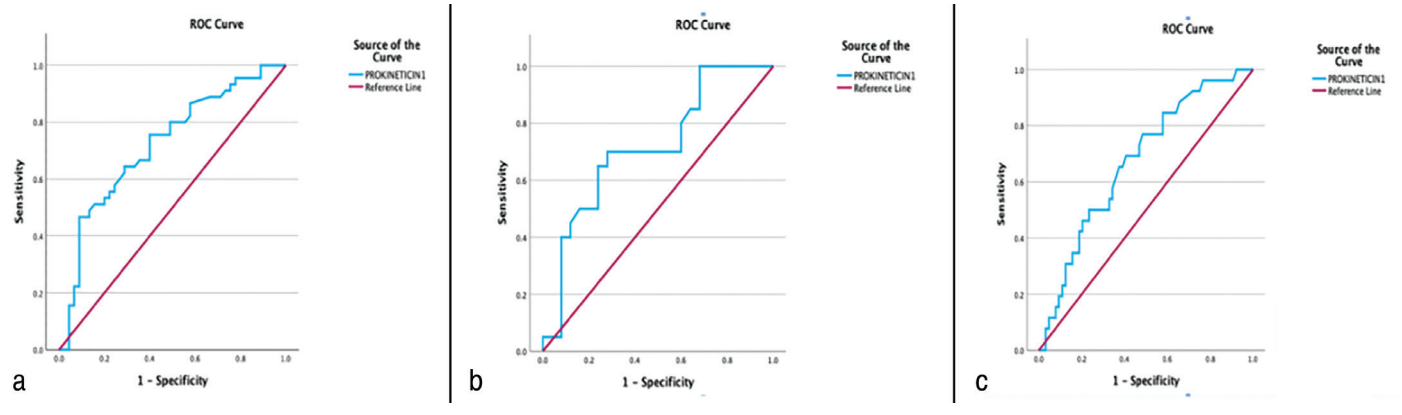

Figure 2. Composite receiver operating characteristic (ROC) curves of maternal serum prokineticin-1 levels for: (a) diagnosis of preeclampsia, (b) prediction of composite adverse perinatal outcomes, and (c) prediction of disease severity in preeclampsia

Table 4. Diagnostic and prognostic performance of PROK-1 for preeclampsia, adverse perinatal outcomes, and disease severity

	Cut-off*	Sensitivity	Specificity	AUC	%95 CI	p-value
Preeclampsia	>5.40	75.6%	60.0%	0.721	0.61-0.82	**<0.001**
Composite adverse perinatal outcomes	>6.53	65.5%	62.5%	0.673	0.55-0.79	**0.004**
Severe preclampsia	>9.80	70.0%	72.0%	0.716	0.56-0.86	**0.006**
*Cut-off values were found according to Youden index. AUC: Area under the curve, CI: Confidence Interval						

to inadequate vascular remodeling, a hallmark of PE. An experimental animal study reported that prolonged PROK-1 overexpression caused pregnancy-related hypertension (21), suggesting that sustained upregulation may contribute directly to PE pathogenesis. In line with these findings, our observation of higher maternal PROK-1 levels in diagnosed PE cases may provide indirect evidence of an earlier pathogenic role.

Previous studies also indicated that alterations in PROK-1 expression may influence pregnancy outcomes. Hoffman et al. (18) demonstrated that PROK-1 plays a central role in normal placentation, with significantly higher maternal levels observed in pregnancies affected by PE compared with healthy controls. Similarly, Inan and colleagues found that elevated first-trimester PROK-1 concentrations predicted subsequent PE and fetal growth restriction, whereas lower levels were linked to spontaneous preterm birth and gestational diabetes (22). Taken together, these findings highlight the importance of balanced PROK-1 activity for normal placental development, as both deficiency and excess may disturb homeostasis and contribute to obstetric complications.

However, not all investigations have arrived at uniform conclusions. Ulu et al. (19) found no significant difference in PROK-1 levels between preeclamptic and healthy pregnancies, while a trend towards elevated values was noted in severe PE. The study indicated that PROK-1 levels were generally lower in the mild PE cohort than in the severe PE cohort, but this difference was not significant (19). Differences in study design, patient selection, timing of sample collection, and assay sensitivity may explain these discrepancies. In our cohort, the higher PROK-1 concentrations among severe PE cases align more closely with the pathophysiologic mechanisms proposed in experimental data, supporting the hypothesis that PROK-1 upregulation may reflect endothelial and placental dysfunction.

Our results also revealed a significant association between PROK-1 and CAPO. Mechanistically, PROK-1 influences endothelial proliferation and vascular remodeling through specific PROK receptors, and its expression is enhanced under hypoxic conditions (23,24). These pathways may explain how elevated PROK-1 levels contribute to placental insufficiency and adverse fetal outcomes or may reflect the hypoxia present in palcenta and supplying vessels in existing PE. Due to the observational nature of our study, causal inference cannot be established. Further prospective, mechanistic and longitudinal research is required to determine whether PROK-1 acts as a driver of pathology or as a compensatory response to placental hypoxia.

From a diagnostic perspective, PROK-1 demonstrated moderate accuracy for distinguishing PE from normal pregnancy (AUC =0.721) and fair ability to predict severe disease and CAPO. Although its independent diagnostic performance remains limited, PROK-1 may enhance accuracy as part of a multimarker panel. Current evidence supports the utility of angiogenic factors, such as soluble fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PlGF) in PE prediction and monitoring (8,25). The combination of a high sFlt-1/PlGF ratio with other emerging angiogenic markers, including PROK-1, could potentially improve predictive accuracy and clinical decision-making.

These findings highlight the potential role of PROK-1 as an adjunctive biomarker that may contribute to earlier risk stratification and improved clinical decision-making in pregnancies at risk for PE.

Study limitations

A key strength of our study lies in its prospective design and well-matched control group. Inclusion of both early- and LO-PE subtypes allowed subgroup comparisons and yielded consistent results across gestational stages. Moreover, beyond evaluating diagnostic potential, we also assessed PROK-1's association with disease severity and neonatal outcomes which added a prognostic dimension rarely explored in prior studies. Nonetheless, specific limits must be recognized. The study sample was modest and sourced from a single center, constraining generalizability. Serum PROK-1 levels were measured only at the time of diagnosis, preventing evaluation of temporal trends or early predictive value. Furthermore, given that PROK-1 alone provided only moderate diagnostic strength, it should likely be interpreted alongside other biochemical and clinical markers rather than as a stand-alone test.

Conclusion

Maternal serum PROK-1 levels were significantly elevated in pregnancies complicated by diagnosed PE and correlated with both disease severity and adverse perinatal outcomes. Although, diagnostic performance in isolation was moderate, PROK-1 may serve as a complementary marker when combined with established angiogenic biomarkers, such as sFlt-1 and PlGF. Integrating PROK-1 into multimarker predictive models may enhance the early identification and risk stratification of PE. Future multicenter and longitudinal studies with early gestational sampling that continues throughout pregnancy until delivery are needed to confirm clinical applicability and temporal changes throughout pregnancy, both normal and when affected by PE. This information would help to clarify whether elevated PROK-1 represents a causal factor or a secondary response to placental dysfunction.

Ethics

Ethics Committee Approval: *The study protocol was approved by the University of Health Sciences Türkiye, Ankara Etlik City Hospital Ethics Committee (approval number: 2024-252, date: 03.04.2024), and all procedures adhered to the Declaration of Helsinki.*

Informed Consent: *All women had signed an informed consent that their data may be used in research studies.*

Footnotes

Author Contributions: *Surgical and Medical Practices: M.A.O., A.K., İ.A., K.Y.Y., Concept: M.A.O., K.Y.Y., Design: M.A.O., A.K., K.Y.Y., Data Collection or Processing: M.A.O., A.K., İ.A., K.Y.Y., Analysis or Interpretation: M.A.O., A.K., İ.A., Literature Search: M.A.O., A.K., İ.A., Writing: M.A.O.*

Conflict of Interest: *No conflict of interest is declared by the authors.*

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Meta-analysis of changes in epithelial ovarian cancer incidence rates associated with salpingectomy: A comparison of 2022–2023 and earlier periods

Greg Marchand[1], Daniela Gonzalez Herrera[1], Brooke Hamilton[1], McKenna Robinson[1], Emily Kline[1], Sarah Mera[1], Michelle Koshaba[1], Greenley Jephson[1], Nidhi Pulicherla[1], Ali Azadi[2]

[1]Marchand Institute for Minimally Invasive Surgery, Arizona, United States of America

[2]Department of Obstetrics and Gynecology, University of Arizona Faculty of Medicine, Arizona, United States of America

Abstract

This systematic review and meta-analysis evaluated epithelial ovarian cancer (EOC) incidence rates associated with salpingectomy, with an exploratory assessment of temporal trends following guideline-driven increases in opportunistic salpingectomy. A literature search was conducted across PubMed, Web of Science, Cochrane, and Scopus, targeting cohort studies published between January 2015 and September 2023. Eligible studies were cohort studies reporting EOC incidence in women undergoing salpingectomy (opportunistic or risk-reducing) compared to controls without salpingectomy, with sufficient sample sizes (>100 salpingectomy cases) and homogeneous populations. The Newcastle-Ottawa Scale (NOS) was used to assess methodological quality. A meta-analysis calculated the risk ratio (RR) of EOC incidence, with subgroup analysis exploring temporal trends, using a random-effects model. Five cohort studies, involving 5,819,102 women with 31,586 EOC cases, were included, all with low risk-of-bias (NOS scores ≥6). Salpingectomy was associated with a 77.7% reduction in EOC incidence compared to control patients [RR =0.223, 95% confidence interval: (0.182, 0.274), p<0.001; I^2=0]%. The 2022-2023 period should be interpreted as a recent evidence window rather than a formally powered comparative period owing to the small number of studies available. These findings confirm that salpingectomy, particularly opportunistic procedures, substantially reduces EOC incidence. Clinicians should consider offering salpingectomy to average-risk women during gynecologic surgeries, with informed consent. Further research with longer follow-up of contemporary cohorts is needed. [J Turk Ger Gynecol Assoc.2026; 27(1): 51-60]

Keywords: EOC, epithelial cell ovarian cancer, meta-analysis, RRSO, salpingectomy

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Introduction

Epithelial ovarian cancer (EOC) remains a significant public health concern, ranking as the sixth leading cause of cancer-related mortality among women worldwide and causing more deaths than any other gynecologic malignancy. High-grade serous carcinoma, the most common EOC subtype, accounts for approximately 85% to 90% of cases and is frequently diagnosed at an advanced stage, which complicates treatment efforts and contributes to elevated mortality rates (1-3). The limited effectiveness of current screening strategies has shifted the focus toward primary prevention strategies, particularly for women at average risk of developing EOC.

Recent research has identified the fallopian tubes as the primary site of origin for many high-grade serous EOC cases, with serous tubal intraepithelial carcinoma (STIC) recognized as a critical precursor lesion (4,5). This discovery has led to increased use of salpingectomy as a preventive strategy, particularly through opportunistic salpingectomy performed during benign

Address for Correspondence: *Greg Marchand*
e-mail: *gm@marchandinstitute.org* ***ORCID:*** *orcid.org/0000-0003-4724-9148*

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gynecologic surgeries or as permanent sterilization (6). Since 2015, the American College of Obstetricians and Gynecologists (ACOG) and other organizations have recommended opportunistic salpingectomy, with reinforcement in 2019, resulting in rising adoption (7). Observational studies have suggested risk reductions of 49-65% with minimal impact on ovarian function (8).

This systematic review and meta-analysis evaluated EOC incidence rates associated with salpingectomy, with an exploratory assessment of temporal trends following the increased adoption of opportunistic salpingectomy after the 2015 and 2019 ACOG Guideline updates. We hypothesized that the protective effect would be consistent across the period, while acknowledging that the limited number of very recent large-scale studies precludes definitive conclusions about further strengthening of the effect in 2022-2023.

Methodology

This review followed PRISMA guidelines (9).

Inclusion and exclusion criteria

To maintain a focused scope on the changes in EOC incidence rates over time, the inclusion criteria were carefully tailored to select high-quality cohort studies that directly address the study's objective. Eligible studies were required to be either retrospective or prospective cohort studies that reported EOC incidence rates in women who underwent salpingectomy, whether performed as an opportunistic procedure (for example, during hysterectomy or sterilization) or as a risk-reducing intervention (such as in women with *BRCA* mutations). To reflect contemporary salpingectomy practices influenced by the 2015 and 2019 guideline updates from the ACOG, studies were required to have been published between January 2015 and September 2023. An exception was made for pivotal studies published before 2015 that included large sample sizes (greater than 10,000 women) and provided essential historical context for understanding earlier EOC incidence trends. In addition, studies were required to have sufficient salpingectomy sample sizes, defined as greater than 100 cases, to ensure adequate statistical power for estimating changes in EOC incidence rates. Studies also needed to focus on homogeneous populations, such as average risk women undergoing opportunistic salpingectomy or clearly defined high-risk groups (for example, *BRCA* mutation carriers), with control groups that were free of confounding surgical interventions, such as hysterectomy, which could obscure the specific effect of salpingectomy on EOC incidence.

Studies were excluded from the analysis if they did not report overall EOC incidence rates, focusing instead on related outcomes, such as the prevalence of STIC or specific histological subtypes of ovarian cancer without providing aggregate EOC incidence data. Randomized controlled trials were excluded, as they were deemed less relevant for capturing observational data on EOC incidence rates in real-world clinical settings. Molecular studies, *in vitro* studies, *in vivo* studies, narrative reviews, and case reports were also excluded due to their inability to provide comparative incidence rate data suitable for meta-analysis. Studies that lacked a control group were not considered, as they could not provide a relative measure of the effect of salpingectomy on EOC incidence. Furthermore, studies with small salpingectomy sample sizes (fewer than 100 cases) or heterogeneous populations, such as those with control groups that included women who had undergone hysterectomy or tubal ligation, were excluded to maintain consistency in population characteristics and reduce potential confounding factors. Studies published before 2015 were generally excluded unless they met the criteria for pivotal studies (n>10,000), ensuring that the included studies were aligned with current clinical practices and guideline-driven salpingectomy adoption.

These inclusion and exclusion criteria were specifically designed to prioritize studies that provide relevant data on the changes in EOC incidence rates over time, ensuring statistical robustness, population homogeneity, and alignment with contemporary salpingectomy practices, thereby facilitating a reliable meta-analysis of temporal trends in EOC incidence.

Information sources and search strategy

A comprehensive literature search was conducted across four major academic databases, which were PubMed, Web of Science, Cochrane, and Scopus, to identify relevant studies published between January 2015 and September 2023. The search strategy was carefully developed to capture studies that address EOC incidence rates in the context of salpingectomy, incorporating a broad range of search terms to maximize the sensitivity of the search. The key search terms included "Epithelial ovarian cancer," "serous tubal intraepithelial carcinoma," "isolated serous intraepithelial carcinoma," "incidental serous tubal intraepithelial carcinoma," "TIC," "Tubal intraepithelial carcinoma," "salpingectom*," "opportunistic salpingectomy," and "risk-reducing salpingectomy." The complete search strategy, including the specific combinations and Boolean operators used, is provided in Supplementary File to ensure reproducibility. To enhance the comprehensiveness of the search, the reference lists of included studies and relevant review articles were manually searched to identify any additional studies that might have been missed by the database searches. Given that this study only used publicly available, previously published data, no institutional review board approval was required for the conduct of this research.

Study selection

The study selection process was conducted in a rigorous, two-stage approach to ensure accuracy and consistency in identifying studies that meet the inclusion criteria. In the first stage, two independent reviewers screened the titles and abstracts of all identified records to eliminate studies that were clearly irrelevant to the research question, using a standardized eligibility form based on the predefined inclusion and exclusion criteria. In the second stage, the full-text articles of potentially relevant studies were retrieved and independently reviewed by the same two reviewers to confirm their eligibility for inclusion in the meta-analysis. Any discrepancies or disagreements between the reviewers during either stage of the screening process were resolved through discussion, and, when necessary, a third reviewer was consulted to achieve consensus. Data management was facilitated using Microsoft Excel, and duplicate records were removed using EndNote software to streamline the screening process and ensure efficiency.

Data extraction

Data extraction was performed independently by two reviewers using a pre-designed data extraction form to ensure consistency and accuracy in capturing the relevant information from each included study. The extracted data included study identification details (such as author names and publication year), country of origin, study design (retrospective or prospective cohort), population characteristics (including whether the population consisted of average risk women or high-risk *BRCA* mutation carriers), exposure details (type of salpingectomy, such as opportunistic or risk-reducing), inclusion and exclusion criteria, the number of EOC cases reported, the total sample size, and risk estimates [such as risk ratios (RRs) or odds ratios]. Any discrepancies identified during the data extraction process were resolved through consensus between the reviewers, ensuring the reliability of the extracted information. This process was designed to capture all data necessary for both the qualitative and quantitative synthesis of the study findings.

Risk-of-bias assessment

The methodological quality of the included studies was assessed using the Newcastle-Ottawa Scale (NOS) for cohort studies, which is a widely recognized tool for evaluating the quality of non-randomized studies (10). The NOS assesses three key domains: selection (including representativeness of the exposed cohort, selection of the non-exposed cohort, ascertainment of exposure, and demonstration that the outcome of interest was not present at the start of the study), comparability (based on the design or analysis to control for confounders), and outcome (including assessment of the outcome, duration of follow-up, and adequacy of follow-up). Each study was scored out of a maximum of nine points, with scores of six or higher indicating a low risk-of-bias. The assessment was conducted independently by two investigators, and any disagreements were resolved through discussion or by consulting a third investigator to ensure consistency and objectivity in the evaluation process.

Data synthesis

Both qualitative and quantitative syntheses were performed. For the quantitative analysis, a meta-analysis calculated the RR of EOC incidence in women undergoing salpingectomy versus controls using a random-effects model. A subgroup analysis was performed to explore temporal trends by separating studies predominantly reflecting pre-2022 practice from the limited number of studies published in 2022-2023. This temporal subgrouping is exploratory in nature due to the small number of studies in the most recent evidence window. Heterogeneity was assessed using I^2; sensitivity analyses explored sources of heterogeneity.

Statistical analysis

All statistical analyses were conducted using Open-Meta Analyst software, which is a robust and widely used tool for performing meta-analytic calculations. A p-value of less than 0.05 was considered statistically significant for all statistical tests. The RR and its corresponding 95% confidence interval (CI) were calculated for each included study and then pooled to estimate the overall effect of salpingectomy on EOC incidence rates across the study periods. The subgroup analysis and sensitivity analyses were designed to enhance the robustness of the findings and to address any potential sources of variability among the included studies.

Results

Study selection

The study selection process is comprehensively illustrated in the PRISMA flowchart (Figure 1), which outlines the steps taken to identify and include relevant studies. A total of 2018 records were initially identified across the four databases searched: PubMed contributed 977 records, Web of Science provided 975 records, Cochrane contributed 58 records, and Scopus yielded 8 records. After removing 201 duplicate records, 1817 unique titles and abstracts were screened for relevance to the study's objective of evaluating changes in EOC incidence rates associated with salpingectomy. During this screening phase, 1801 records were excluded, primarily because they did not address EOC incidence rates, focused on unrelated interventions, or did not involve salpingectomy as the primary exposure. The remaining 16 full-text articles were thoroughly

assessed for eligibility against the predefined inclusion and exclusion criteria.

Ultimately, five cohort studies were included in the final analysis, as they met all criteria, including cohort study design, reporting of EOC incidence rates, sufficient sample size, and population homogeneity. The 11 studies that were excluded were deemed ineligible for these specific reasons: three studies involved mixed populations with confounding surgical interventions, such as hysterectomy or tubal ligation included in the control group, which could obscure the effect of salpingectomy; two studies were excluded due to small salpingectomy sample sizes (fewer than 100 cases) or because they were published before 2015, reflecting outdated clinical practices not aligned with current guideline-driven salpingectomy adoption; one study focused on an irrelevant population that did not meet the study's criteria; and five studies addressed irrelevant interventions or outcomes, such as the prevalence of STIC rather than overall EOC incidence rates. This rigorous selection process ensured that only high-quality studies reporting EOC incidence after salpingectomy were included, allowing an overall meta-analysis and an exploratory examination of temporal trends.

Risk-of-bias

The risk-of-bias assessment for the five included cohort studies was conducted using the NOS, and the results are presented in Table 1, demonstrating that all studies achieved a low risk-of-bias, with NOS scores of 6 or higher. In the selection domain, most studies performed strongly, with clear representativeness of the exposed cohort (women undergoing salpingectomy) and appropriate selection of the non-exposed cohort (controls without salpingectomy). However, the studies by Hanley et al. (11) and Falconer et al. (12) exhibited a higher risk of selection bias due to insufficient detail in describing the participant selection processes, which could potentially introduce bias into the study findings. In the comparability domain, the majority of the studies adequately controlled for key confounders, such as age, genetic risk factors, or other relevant clinical variables, ensuring that the salpingectomy and control groups were comparable at baseline. Nevertheless, Hanley et al. (11) and Falconer et al. (12) demonstrated a higher risk of comparability bias due to limited or inadequate adjustment for confounders, which could affect the reliability of the comparisons between the study groups. The outcome domain was consistently robust across all five studies, with accurate and standardized methods

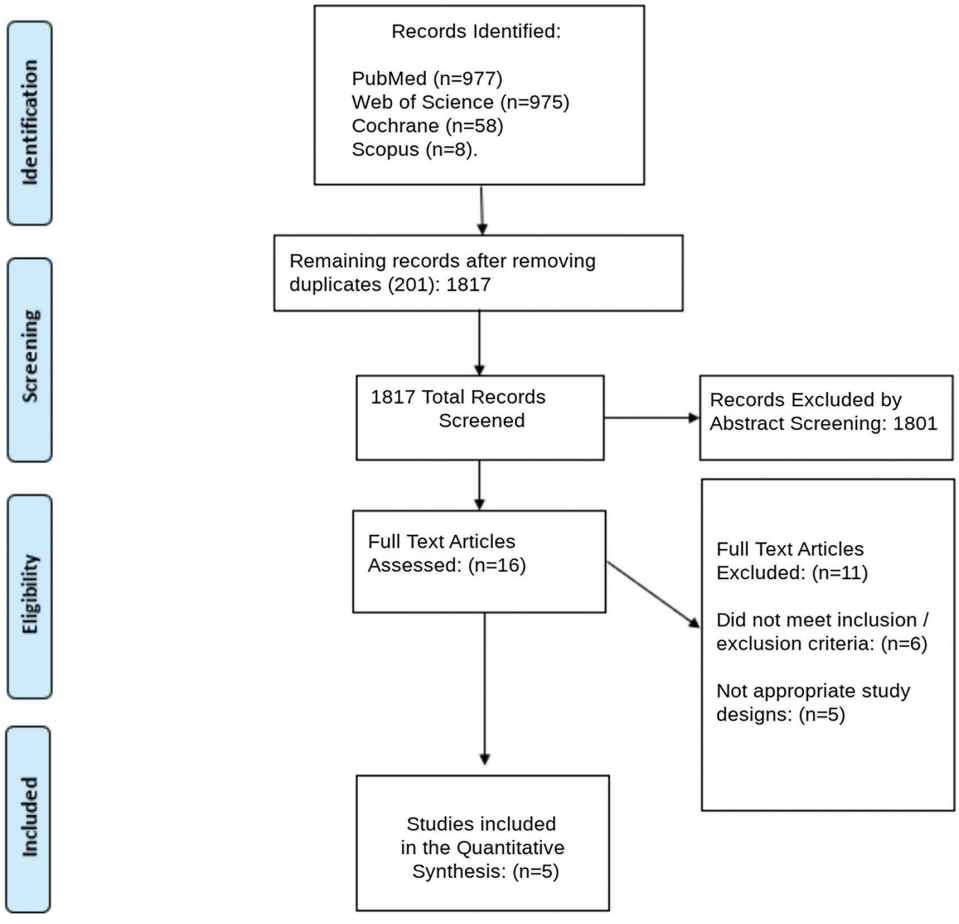

Figure 1. PRISMA flowchart

for assessing EOC incidence, sufficiently long follow-up periods to capture incident cases, and minimal loss to follow-up, ensuring that the outcome data were reliable. The overall low risk-of-bias across the included studies supports the reliability and validity of their findings for inclusion in the meta-analysis, although the noted selection and comparability biases in the studies by Hanley et al. (11) and Falconer et al. (12) suggest that their results should be interpreted with a degree of caution to account for potential methodological limitations.

Study characteristics

The characteristics of the five included cohort studies are summarized in Table 2, providing a detailed overview of the studies that contributed to the meta-analysis. Collectively, these studies involved a total of 5,819,102 women, with 31,586 EOC cases identified across the cohorts, offering a substantial sample size for evaluating changes in EOC incidence rates over time. The studies were conducted in diverse geographical regions, with two studies from Canada, one from the Netherlands, one from England, and one from Sweden, reflecting a predominantly Western population but providing a broad perspective on salpingectomy practices. The included studies encompassed two distinct population types: average risk women undergoing opportunistic salpingectomy, typically performed during benign gynecologic surgeries or as a method of sterilization, and high-risk women, specifically *BRCA1* or *BRCA2* mutation carriers, undergoing risk-reducing salpingo-oophorectomy (RRSO). Giannakeas et al. (13) conducted a retrospective cohort study in Ontario, Canada, involving 32,879 women who underwent salpingectomy, with 31 EOC cases identified during the follow-up period, providing recent data relevant to the 2022-2023 period. Hanley et al. (11) evaluated 25,889 women who underwent opportunistic salpingectomy, reporting only 5 EOC cases, which was significantly fewer than the expected number based on the incidence rates observed in the control group. Blok et al. (14) conducted a prospective cohort study in the Netherlands, focusing on 527 *BRCA1* or *BRCA2* mutation carriers who underwent RRSO, with 12 cases of high-grade serous ovarian cancer identified, often of tubal origin, reflecting the high-risk nature of this population. Stanciu et al. (15) examined a smaller cohort of 287 women in England, primarily *BRCA* mutation carriers or those with a strong family history of breast or ovarian cancer, who underwent RRSO, identifying 1 case of EOC, which developed after 92 months. Falconer et al. (12), a pivotal retrospective cohort study from Sweden, analyzed a large cohort of 5,819,102 women, of which 71,781 underwent salpingectomy, reporting a total of 31,399 EOC cases, with the majority (31,311 cases) occurring in the control group, indicating a reduced incidence rate in the salpingectomy group. These five studies collectively provide a robust dataset for estimating the overall protective effect of salpingectomy and for an exploratory examination of temporal trends.

Synthesis of results

A double-arm random-effects meta-analysis of the three studies in average risk women undergoing opportunistic salpingectomy [Falconer et al. (12), Hanley et al. (11), Giannakeas et al. (13)] yielded a pooled RR of 0.223 (95% CI: 0.182-0.274, $p<0.001$;

Table 1. Newcastle-Ottawa Scale risk-of-bias assessment for included cohort studies

Study	Selection (max 4)	Comparability (max 2)	Outcome (max 3)	Total score (max 9)
Giannakeas et al. (13)	★★★★	★★	★★★	9
Hanley et al. (11)	★★	★	★★★	6
Blok et al. (14)	★★★★	★★	★★★	9
Stanciu et al. (15)	★★★★	★★	★★★	9
Falconer et al. (12)	★★	★	★★★	6

Stars (★) indicate points awarded in each domain. Hanley et al. (11) and Falconer et al. (12) received fewer stars in selection and comparability due to higher risk-of-bias, as described. Total scores ≥6 indicate low risk-of-bias

Table 2. Characteristics of included studies

Study	Country	Design	Population	Salpingectomy type	EOC cases	Sample size
Giannakeas et al. (13)	Canada	Retrospective cohort	Average risk women	Opportunistic	148	131,516
Hanley et al. (11)	Canada	Retrospective cohort	Average risk women	Opportunistic	26	57,969
Blok et al. (14)	Netherlands	Prospective cohort	BRCA1/2 mutation carriers	Risk-reducing (RRSO)	12	527
Stanciu et al. (15)	England	Retrospective cohort	BRCA carriers/high-risk	Risk-reducing (RRSO)	1	287
Falconer et al. (12)	Sweden	Retrospective cohort	Average risk women	Opportunistic	31,399	5,628,803
EOC: Epithelial ovarian cancer, RRSO: Risk-reducing salpingo-oophorectomy						

I^2=0%) after exclusion of Giannakeas et al. (13) in sensitivity analysis to resolve initial heterogeneity (Figure 2).

The 2022-2023 subgroup is presented as an exploratory recent evidence window; it is not a formally powered temporal comparison due to the limited number of studies and shorter follow-up durations in this period. Exploratory temporal subgroup analysis showed a consistent protective effect in the pre-2022 period (RR: 0.220, 95% CI: 0.179-0.272) and a non-significant point estimate in the limited 2022-2023 evidence window (RR: 0.538, 95% CI: 0.208-1.389; I^2=70.7%) (Figure 3). Single-arm incidence in the opportunistic salpingectomy subgroup was 0.001 (95% CI: 0.001-0.001) after sensitivity analysis (Figure 4).

Discussion

The exploratory temporal subgroup analysis does not provide evidence of a statistically stronger protective effect in the most recent 2022-2023 evidence window compared with earlier years, which is expected, given the still-limited number of large contemporary cohorts with sufficient follow-up (Figure 5). To the best of our knowledge, this remains one of the few meta-analyses to quantitatively synthesize the rapidly accumulating observational evidence on opportunistic salpingectomy in average risk women.

The protective effect of salpingectomy on EOC incidence is biologically plausible and well-supported by the understanding that the fallopian tubes serve as the primary site of origin for many high-grade serous EOC cases, with STIC identified as a key precursor lesion (5,6). By removing the fallopian tubes, salpingectomy effectively eliminates the anatomical site where STIC develops, thereby interrupting the oncogenic pathway that leads to EOC. This mechanism is reflected in the findings of the included studies, particularly Falconer et al. (12), which reported a 65 percent reduction in ovarian cancer risk primarily occurring in the control group, indicating a significant protective effect of salpingectomy (10). Similarly, Hanley et al. (11) observed only 5 EOC cases among 25,889 women in the salpingectomy group, a number far below the expected incidence based on control group rates, further supporting the preventive impact of opportunistic salpingectomy (11). In contrast, Giannakeas et al. (13) reported no significant association between salpingectomy and EOC incidence among 32,879 women, with only 31 EOC cases identified, which may be attributed to a short follow-up period and low event rates that limited the study's statistical power to detect a significant

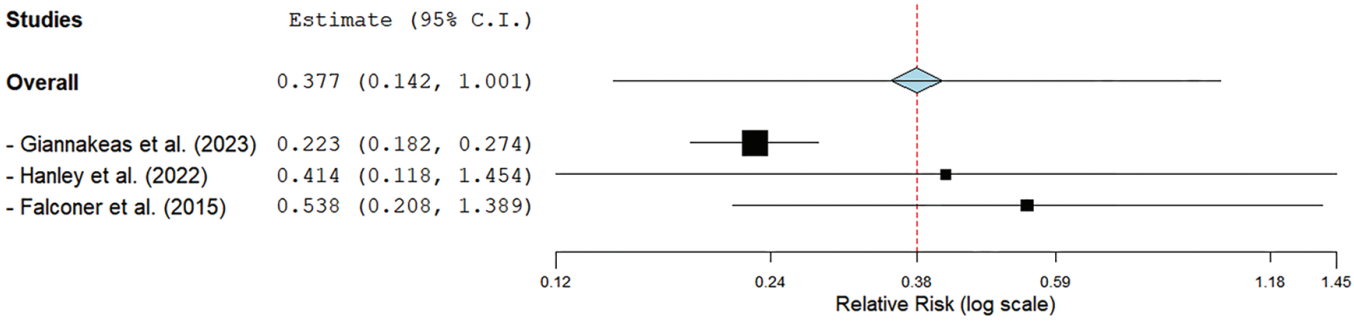

Figure 2. Leave-one-out test of the double-arm meta-analysis model of EOC incidence
EOC: Epithelial ovarian cancer, C.I.: Confidence interval

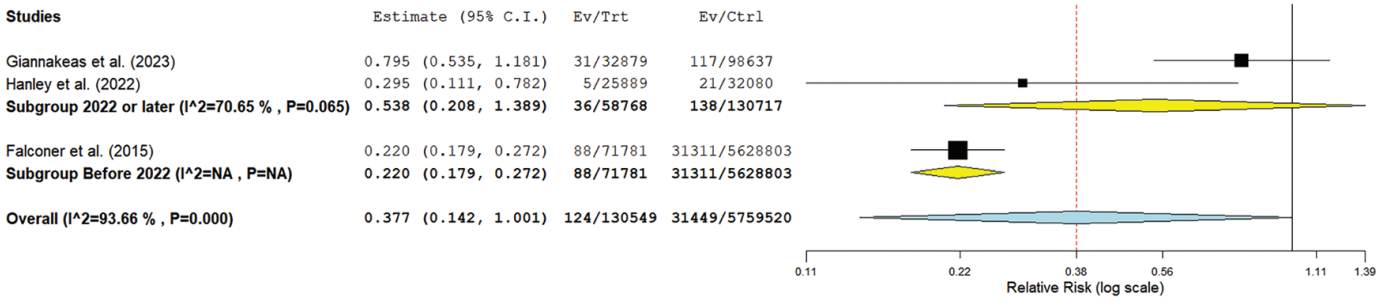

Figure 3. Subgroup analysis based on year for the double-arm meta-analysis model of EOC incidence
EOC: Epithelial ovarian cancer, C.I.: Confidence interval

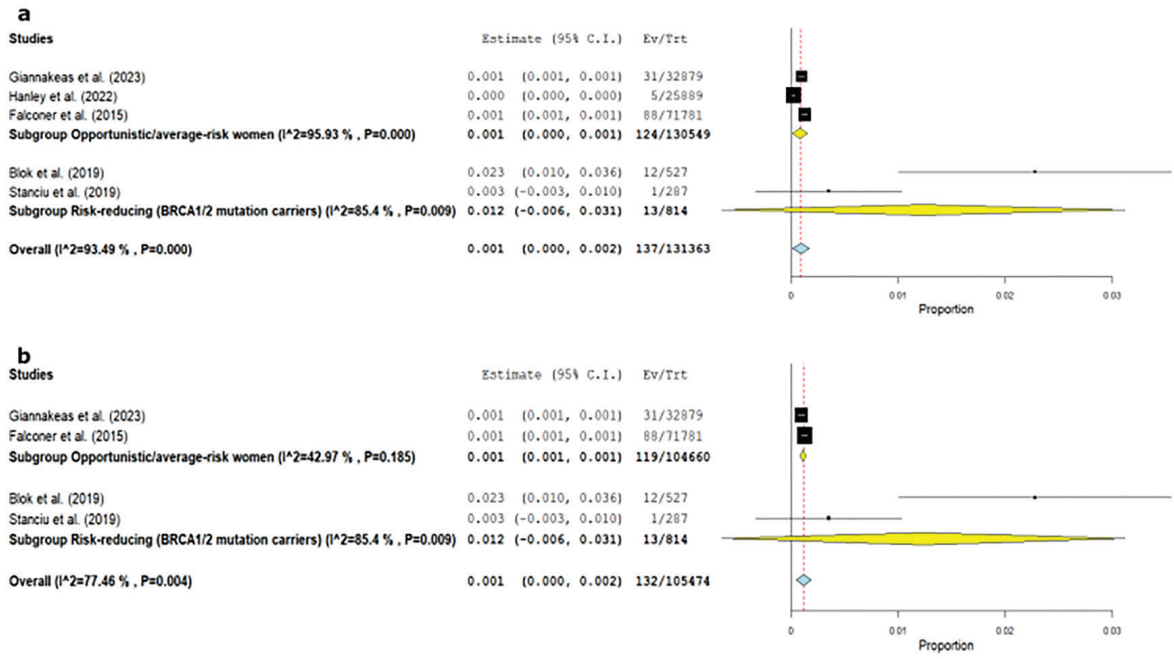

Figure 4. (a) Single-arm meta-analysis of EOC incidence with a subgroup analysis based on approach to salpingectomy and BRCA1/2 mutation status and (b) single-arm meta-analysis of EOC incidence with sensitivity analysis of the opportunistic approach subgroup

EOC: Epithelial ovarian cancer, C.I.: Confidence interval

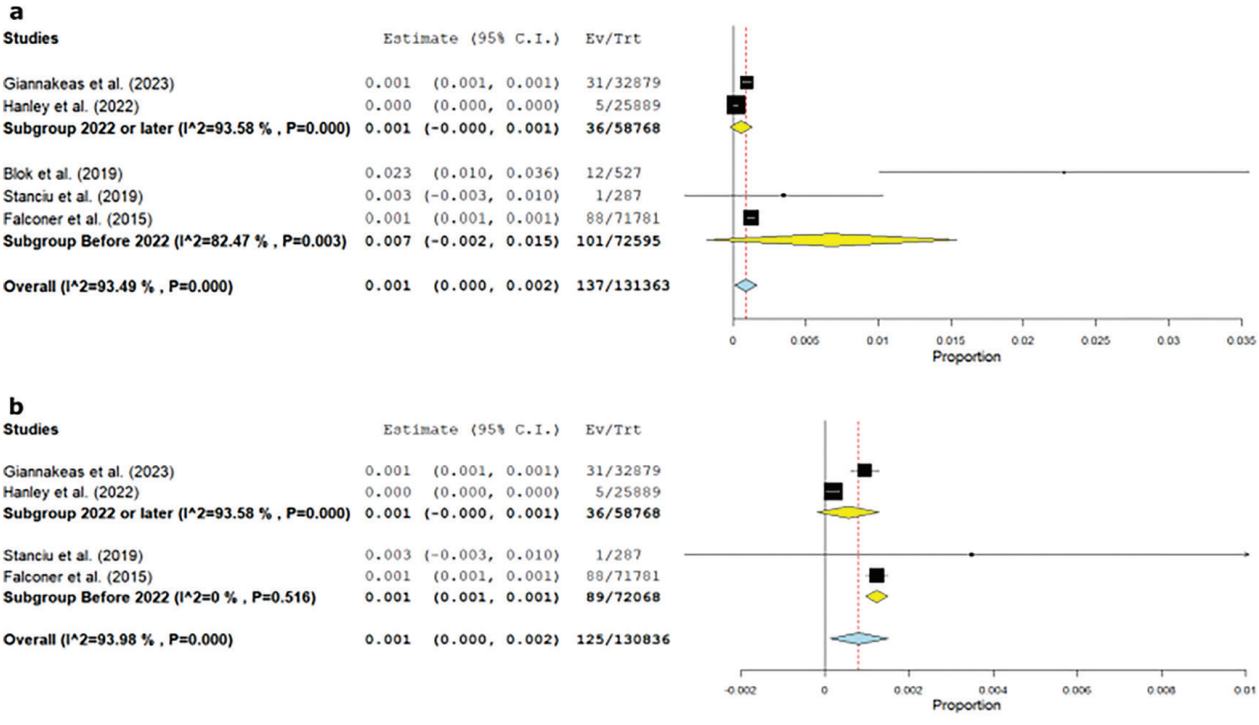

Figure 5. (a) Single-arm meta-analysis of EOC incidence with a subgroup analysis based on year and (b) single-arm meta-analysis of EOC incidence with sensitivity analysis of the subgroup of pre-2022

EOC: Epithelial ovarian cancer, C.I.: Confidence interval

effect (12). The studies by Blok et al. (14), which included 527 *BRCA* mutation carriers with 12 EOC cases, and Stanciu et al. (15), which included 287 *BRCA* mutation carriers with one EOC case developed during follow-up, provide evidence of salpingectomy's protective effect in high-risk populations, although their smaller sample sizes contributed less to the overall pooled effect compared to the larger studies focused on average risk women (13,14).

Study Limitations

This study possesses several notable strengths that enhance its scientific rigor and reliability, making it, we believe, a valuable contribution to the field of gynecologic oncology. The comprehensive literature search conducted across four major academic databases ensured a thorough and systematic identification of relevant studies, minimizing the risk of missing key publications that address EOC incidence rates in the context of salpingectomy. The inclusion of only high-quality cohort studies, all of which demonstrated a low risk-of-bias as confirmed by NOS scores of 6 or higher, bolsters the credibility and validity of the findings, ensuring that the meta-analysis is based on robust and reliable data. The large total sample size of 5,819,102 women, with 31,586 EOC cases identified across the five studies, provides substantial statistical power for the overall meta-analysis of the protective effect of salpingectomy, enhancing confidence in the study's results. The specific focus on comparing EOC incidence rates in 2022-2023 to earlier periods aligns closely with the recent guideline-driven changes in clinical practice, particularly the increased adoption of opportunistic salpingectomy, making the findings highly relevant to current clinical contexts and public health strategies. In addition, the use of a random-effects model in the meta-analysis, combined with sensitivity analyses to explore sources of heterogeneity, ensured a comprehensive and robust assessment of the effect of salpingectomy on EOC incidence rates, accounting for variability across studies and populations. Despite its strengths, this study is subject to several limitations that warrant careful consideration when interpreting the findings. The high level of heterogeneity observed in the meta-analysis, as indicated by I^2 values, suggests significant variability in the effect sizes reported across the included studies, which may impact the precision of the pooled RR estimate. This heterogeneity is likely attributable to differences in the study populations, with three studies focusing on average risk women undergoing opportunistic salpingectomy and two studies focusing on high-risk *BRCA* mutation carriers undergoing RRSO, as well as variations in the indications for salpingectomy, such as opportunistic versus risk-reducing procedures. Sensitivity analyses and subgrouping analyses helped to resolve heterogeneity in some, but not all subgroups, which suggests that population differences are a significant contributor to the heterogeneity. The remaining residual variability indicates the need for cautious interpretation of the results. The reliance on observational cohort studies, rather than randomized controlled trials, limits the ability to establish a causal relationship between salpingectomy and reduced EOC incidence rates, as unmeasured confounders, such as differences in healthcare access or underlying risk factors, may have influenced the observed associations. The predominance of studies conducted in Western countries, specifically Canada, the Netherlands, England, and Sweden, may restrict the generalizability of the findings to other populations, particularly those in low-resource settings or regions with different healthcare practices and salpingectomy adoption rates. By prioritizing studies published after 2015 with sufficient sample sizes and homogeneous populations, the study may have excluded earlier studies or those with smaller sample sizes, potentially limiting the historical context for understanding long-term trends in EOC incidence rates, although the inclusion of the pivotal study by Falconer et al. (12) mitigates this to some extent. The low number of EOC cases reported in some studies, such as Hanley et al. (11) with only 5 cases among 25,889 women and Stanciu et al. (15) with one case among 287 women, may have reduced the precision of the effect estimates for these studies, although the large sample sizes of other studies, such as Falconer et al. (12) with 5,628,803 women and Giannakeas et al. (13) with 131,516 women, compensated for this limitation by providing substantial statistical power. Finally, the short follow-up periods in some studies, particularly Giannakeas et al. (13), may have underestimated the true effect of salpingectomy on EOC incidence rates, as EOC may develop years after the procedure, potentially attenuating the observed associations in studies with limited follow-up duration. The 2022-2023 evidence window remains limited in both number of studies and duration of follow-up and therefore does not yet allow definitive conclusions about further strengthening of the protective effect over time.

Clinical implications

The findings of this meta-analysis, which demonstrate a 77.7% reduction in EOC incidence rates associated with salpingectomy compared to control patients, have significant implications for clinical practice, particularly in the field of gynecologic surgery. The substantial reduction in EOC incidence rates, supported by a large sample, provides strong evidence to support the use of opportunistic salpingectomy as a primary prevention strategy for average risk women undergoing benign gynecologic surgeries, such as hysterectomy or sterilization. Clinicians should engage in shared decision-making with their patients, discussing the significant protective benefits of salpingectomy in reducing EOC incidence rates, which is particularly relevant

given the high mortality associated with this malignancy, alongside the potential risks of the procedure, such as surgical complications or permanent infertility, which may be a concern for women who have not completed childbearing. The continued endorsement of opportunistic salpingectomy in professional guidelines, such as those issued by ACOG, is warranted based on these findings, and healthcare providers should advocate for the integration of salpingectomy into routine clinical practice where appropriate, particularly as a replacement for tubal ligation in women seeking permanent sterilization (7). For high-risk populations, such as women with *BRCA1* or *BRCA2* mutations, RRSO remains the standard of care (4), but the findings from studies such as Blok et al. (14) and Stanciu et al. (15), which reported reduced EOC incidence in *BRCA* carriers undergoing salpingectomy, suggest that salpingectomy alone may offer a significant protective effect, potentially serving as a less invasive option for some high-risk women, although further research is needed to confirm this approach. It is important to support the ongoing education and training for gynecoloy surgeons to ensure that salpingectomy is performed safely and effectively, maximizing its preventive potential while minimizing associated risks.

Research implications

The results of this meta-analysis highlight several critical avenues for future research to build upon the current evidence base and further elucidate the impact of salpingectomy on EOC incidence rates over time. Randomized controlled trials are urgently needed to establish a causal relationship between salpingectomy and reduced EOC incidence rates, addressing the inherent limitations of observational cohort studies, which are susceptible to confounding and selection biases. Such trials could provide definitive evidence to guide clinical recommendations and strengthen the case for widespread adoption of salpingectomy as a preventive strategy. Long-term cohort studies with extended follow-up periods are essential to assess the durability of the protective effect of salpingectomy on EOC incidence rates, particularly given that some studies, such as Giannakeas et al. (13), may have underestimated the effect due to short follow-up durations that did not allow sufficient time for EOC cases to manifest. These long-term studies should also evaluate the impact of salpingectomy on other health outcomes, such as overall mortality and quality of life, to provide a comprehensive understanding of its benefits and potential risks. Cost-effectiveness analyses are another critical area for future research, as they would help to evaluate the economic feasibility of implementing widespread salpingectomy programs, particularly in healthcare systems with limited resources, where the costs of surgical interventions must be balanced against their preventive benefits. Moreover, studies conducted in diverse populations, including those from non-Western countries and underrepresented regions, are necessary to enhance the generalizability of the findings, as the current meta-analysis is limited by the predominance of studies from Western countries, which may not fully reflect global variations in healthcare practices and salpingectomy adoption rates. Further research should also explore the optimal timing and indications for salpingectomy, particularly in younger women or those with specific genetic risk profiles, to refine clinical recommendations and maximize the preventive benefits of the procedure while minimizing potential adverse effects. By addressing these research priorities, the medical and scientific community can further solidify the role of salpingectomy in reducing EOC incidence rates and contribute to the development of targeted prevention strategies that have the potential to significantly alleviate the global burden of this deadly malignancy.

Conclusion

Salpingectomy is associated with a substantial and consistent reduction in EOC incidence across the studied period. The currently available data from 2022-2023 do not yet demonstrate a further strengthening of this protective effect, but continued surveillance and longer follow-up of contemporary cohorts are warranted. Clinicians are strongly encouraged to offer opportunistic salpingectomy to average risk women during benign gynecologic surgeries, ensuring informed consent discussions clearly outline the substantial benefits of EOC risk reduction alongside potential risks.

Footnotes

Author Contributions: Surgical and Medical Practices: G.M., A.A., N.P., S.M., M.K., Concept: G.M., A.A., Design: G.M., A.A., D.G.H., B.H., Data Collection or Processing: D.G.H., B.H., M.R., N.P., E.K., M.K., G.J., Analysis or Interpretation: G.M., A.A., D.G.H., Literature Search: D.G.H., B.H., M.R., E.K., Writing: G.M., S.M., M.K., G.J.

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Severe thrombocytopenia and neurological symptoms in pregnancy: a diagnostic challenge between hemolysis, elevated liver enzymes, and low platelet syndrome and thrombotic thrombocytopenic purpura-what is your diagnosis?

A 24-year-old primigravid woman at 35 weeks and 5 days of gestation presented to the emergency service with uterine contractions. Due to persistent contractions, she was admitted to the obstetrics and gynecology ward. Her medical history included hypothyroidism, for which she was taking levothyroxine and she had no prior surgical history.

On examination, her vital signs were stable: blood pressure was 128/78 mmHg; heart rate 84 bpm; and temperature 36.6 °C. Physical examination revealed 2+ pitting edema in both lower extremities. Obstetric ultrasonography showed a singleton live fetus in cephalic presentation with biometric measurements corresponding to 36-37 weeks of gestation and an amniotic fluid index of 16 cm. Non-stress testing revealed irregular uterine contractions, and the cervical length was measured at 33 mm.

Initial laboratory tests demonstrated a hemoglobin level of 8 g/dL, hematocrit 24%, platelet count of 6,000/μL, aspartate aminotransferase 77 IU/L, alanine aminotransferase 31 IU/L, creatinine 1.25 mg/dL, urea 63 mg/dL, lactate dehydrogenase 1407 U/L, and 2+ proteinuria on urinalysis.

Answer

These findings led to a presumed preliminary diagnosis of hemolysis, elevated liver enzymes and low platelets (HELLP) syndrome. The patient was referred to hematology due to severe thrombocytopenia. As a result of the peripheral smear analysis performed by hematology, the platelet value was consistent with the whole blood result, erythrocytes were evaluated as hypochromic microcytic, and schistocytes were not observed. During the physical examination of the patient, petechiae in the places under pressure, laboratory values upon the follow-up of mucosal bleeding and immune thrombocytopenic purpura (ITP) were considered as a differential preliminary diagnosis. Given the absence of schistocytes or abnormal cells on peripheral blood smear, bone marrow examination was deferred. No evidence of infection, drug-induced thrombocytopenia, or autoimmune disease was identified. This supported the preliminary diagnosis of ITP.

With the recommendation of hematology, the patient started treatment with intravenous immunoglobulin (IVIG) at 40/mg/kg/day for 5 days and a single dose of 80 mg of methylprednisolone. After platelet elevation elective birth induction was planned.

On the second day of hospitalization, the patient developed sudden-onset aphasia. Due to concerns of worsening preeclampsia or HELLP syndrome with neurological involvement, an emergency Cesarean section was performed. Preoperative platelet count was 20,000/μL. A live infant weighing 2480 grams was delivered.

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Address for Correspondence: *Halenur Öner Soy*
e-mail: *drhalenuronersoy@gmail.com* ***ORCID:*** *orcid.org/0009-0005-6413-0677*

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Neurology consultation was obtained postoperatively. Brain magnetic resonance imaging (MRI) with diffusion sequences, electroencephalogram, and carotid Doppler ultrasound were all unremarkable. The patient's normal brain MRI ruled out the possibility of ischemic stroke, and the absence of hypertension or seizures ruled out the possibility of preeclampsia and eclampsia.

After five days of IVIG, platelet count increased to 58,000/μL. On postoperative day 5, the patient was discharged with low molecular weight Heparin and instructions for bleeding precautions.

At routine follow-up, she reported blurred vision. Laboratory testing revealed a platelet count of 17,000/μL. This time, the peripheral smear showed schistocytes. ADAMTS13 activity was found to be <10%, confirming the diagnosis of thrombotic thrombocytopenic purpura (TTP). Plasma exchange therapy was initiated immediately.

This case illustrates the diagnostic challenge of thrombocytopenia in pregnancy, where HELLP syndrome, ITP and TTP can present with overlapping features. Initially, HELLP syndrome was suspected due to the combination of hemolysis leading to anemia, elevated liver enzymes, and thrombocytopenia, but the presence of mucocutaneous bleeding and isolated severe thrombocytopenia without schistocytes made ITP more likely. However, the sudden onset of a focal neurologic deficit (aphasia) on the second day of hospitalization raised concern for TTP. An ischemic stroke was considered, but the brain MRI was unremarkable, and eclampsia was deemed less likely in the absence of severe hypertension or seizures. The diagnosis of TTP was confirmed when a repeat peripheral blood smear revealed schistocytes and ADAMTS13 activity <10%. This chronological evolution illustrates that TTP can initially mimic ITP or HELLP syndrome, and that the hallmark of TTP of microangiopathic features may only become evident over time.

HELLP syndrome is a pregnancy or postpartum condition characterized by hemolysis (with microangiopathic findings on peripheral smear), elevated liver enzymes, and thrombocytopenia (1). Although often considered a severe form of preeclampsia, about 15-20% of HELLP cases occur without prior hypertension or proteinuria, suggesting it may be a distinct disorder (2). There is no effective medical treatment to regress HELLP syndrome but delivery may be associated with improvement in clinical signs and symptoms over time. Maternal complications are often hemorrhagic and may include life-threatening hepatic hemorrhage (3). Neonatal outcomes are mainly related to gestational age at delivery, as HELLP frequently results in preterm birth (4).

TTP is a rare, life-threatening thrombotic microangiopathy caused by ADAMTS13 deficiency, which cleaves von Willebrand factor. The traditional diagnostic pentad of fever, thrombocytopenia, microangiopathic hemolytic anemia, neurologic symptoms, and renal failure is now considered outdated, as fewer than 10% of acute cases present with all five features (5). Nearly all patients show severe thrombocytopenia ($<30\times10^3/\mu$L) and microangiopathic hemolytic anemia with schistocytes on peripheral smear. Common symptoms include mucocutaneous bleeding, fatigue, and dyspnea (6).

Symptoms related to organ ischemia or infarction are most commonly neurologic. About 60% of patients present with neurological manifestations, ranging from headache and confusion to stroke, coma, or seizures (7). Furthermore, renal involvement usually manifests as isolated proteinuria or hematuria with acute renal failure being uncommon in TTP, and serum creatinine is typically below 2 mg/dL at presentation. However, acute renal injury does not exclude a diagnosis of TTP. Some studies have reported severe TTP with ADAMTS13 activity <10%, acute kidney injury in 10-27% of patients (8).

HELLP syndrome and TTP are two important conditions that must be considered in the differential diagnosis of preeclampsia, although they have distinct clinical and

Table 1. Comparison of clinical features

Feature/condition	Preeclampsia	HELLP syndrome	TTP	ITP	Present case
Timing	>20 weeks of gestation	Usually 3rd trimester or postpartum	Rare in pregnancy, often postpartum	Can occur anytime	35+5 weeks at admission, diagnosis postpartum
Blood pressure	Elevated (≥140/90 mmHg)	May be elevated; 15-20% normotensive	Usually normal	Normal	128/78 mmHg (normal)
Proteinuria	Present	Often present	Possible (minimal)	Absent	2+ proteinuria

Table 1. Continued

Feature/condition	Preeclampsia	HELLP syndrome	TTP	ITP	Present case
Neurological symptoms	Eclampsia (seizures)	Rare, severe cases	Common (~60%): headache, confusion, stroke	Very rare	Aphasia developed
Treatment	Antihypertensives, $MgSO_4$, delivery	Urgent delivery	Plasma exchange, steroids	IVIG, steroids	Started on IVIG + steroids; plasma exchange added
Postpartum course	Usually resolves	Improves after delivery	Requires continued postpartum treatment	May persist or recur	Initially thought ITP, ultimately TTP confirmed

TTP: Thrombotic thrombocytopenic purpura, ITP: Immune thrombocytopenic purpura, IVIG: Intravenous immunoglobulin, HELLP: Hemolysis, elevated liver enzymes and low platelets

Table 2. Comparison of hematological and laboratory features

Feature/condition	Preeclampsia	HELLP syndrome	TTP	ITP	Presented case
Thrombocytopenia	Mild to moderate	Marked (<100,000/μL)	Severe (<30,000/μL)	Severe (<20,000/μL)	6,000 → 58,000 → 17,000/μL
Hemolysis (anemia)	Rare	Microangiopathic hemolysis (schistocytes)	Severe MAHA (schistocytes, ↑LDH)	Rare; autoimmune if present	Initially no schistocytes → later positive; Hb: 8 g/dL
Liver enzymes	Normal or mildly elevated	AST, ALT, LDH significantly ↑	LDH ↑; AST/ALT usually normal	Normal	AST: 77 IU/L, ALT: 31 IU/L, LDH: 1407 U/L
Renal function	Mild to moderate impairment	Can be impaired	Creatinine <2 mg/dL typical	Normal	Creatinine: 1.25 mg/dL, Urea: 63 mg/dL
Peripheral smear	Usually normal	Schistocytes (+)	Schistocytes (+)	No schistocytes	Initial smear: no schistocytes → later positive
ADAMTS13 activity	Normal	Normal	<10%	Normal	<10% → TTP diagnosis

HELLP: Hemolysis, elevated liver enzymes and low platelets, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, LDH: Lactate dehydrogenase, TTP: Thrombotic thrombocytopenic purpura, ITP: Immune thrombocytopenic purpura

laboratory features. HELLP is typically associated with elevated liver enzymes, hypertension, and proteinuria, whereas TTP is characterized by severe hemolytic anemia, marked thrombocytopenia, neurological symptoms, and ADAMTS13 deficiency. Differentiating between the two is essential for accurate diagnosis and timely intervention. While HELLP syndrome usually resolves after delivery without requiring additional treatment, TTP necessitates continued therapy in the postpartum period (9).

Diseases that should be considered in differential diagnosis are compared in Tables 1 and 2.

As demonstrated in the present case, TTP should remain a consideration in pregnant patients who develop neurological symptoms, even an isolated deficit such as aphasia, since such a presentation might otherwise be attributed to eclampsia or stroke. Accurate differentiation between conditions with similar clinical features is essential to ensure timely initiation of appropriate treatment.

Halenur Öner Soy, Hasan Süt, Özhan Özdemir

Department of Obstetrics and Gynecology, University of Health Sciences Türkiye, Gülhane Faculty of Medicine, Ankara, Türkiye

Footnotes

Conflict of Interest: *No conflict of interest is declared by the authors.*

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A rare case of pyomyoma following hysterotomy in a premenopausal woman with leiomyoma

Dear Editor,

Leiomyomas are benign uterine tumors that occur in up to 75% of women and complicate about 2-10% of pregnancies (1). In rare cases, a leiomyoma may undergo necrosis and become infected, and this condition is referred to as pyomyoma. This condition is associated with high rates of morbidity and may lead to death (2). Herein, we present a case of pyomyoma in a 29-year-old woman with a history of hysterotomy, which was managed by uterus-preserving surgery, as preserving her fertility was important.

A 29-year-old female P0L0A1 presented to the emergency department of obstetrics and gynecology with complaints of severe abdominal pain and high-grade fever for two months. She had a history of hysterotomy performed two months previously in an outside facility**,** for failed medical termination of pregnancy at mid-trimester gestation. On general examination, she was conscious and oriented, with a blood pressure of 80/40 mmHg, heart rate of 134/minute, RR-24/minute, temperature 103.3 °F and saturation 99% on room air with qSOFA of 2/3. On systemic examination, her respiratory and cardiovascular systems were found to be normal. On per abdominal examination, there was no organomegaly, but her uterus was approximately 20 weeks in size and tender. Per speculum examination revealed no foul-smelling discharge, and her cervix and vagina appeared to be grossly normal. Per vaginal examination also indicated that the uterus was approximately 20 weeks in size and exhibited cervical motion tenderness. Her hemoglobin level was 9.2 g/dL, with a total leukocyte count of 31,000/μL and a C-reactive protein (CRP) level of 21.3 mg/dL. Sonography suggested the presence of a heterogeneous mass with echogenic foci of size 10x9.6x9.8 cm with pelvic collection (Figure 1). Management was started for septic shock, including administering a fluid bolus of 1.5 litres. Blood, urine and high vaginal swab culture were taken and empirical antibiotic (piperacillin-tazobactam and teicoplanin) was started. For source control, an emergency exploratory laparotomy was performed. Intraoperatively, an approximately 9x8 cm large low corporeal subserosal fibroid was noted over the posterior wall of the uterus. Approximately 200-300 cc of foul-smelling, purulent fluid was drained from the mass, followed by myomectomy (Figures 2-4). Pus culture was sent, which revealed Gram-negative bacilli. Postoperatively, the patient recovered well with symptomatic improvement. Her white blood cell count and CRP also normalized and she was discharged. Histopathological examination confirmed the diagnosis of suppurative leiomyoma (Figure 5).

Pyomyoma is caused by compromised vascularity, which causes necrosis in leiomyoma and increases its susceptibility to infection (2). Risk factors are submucosal fibroids,

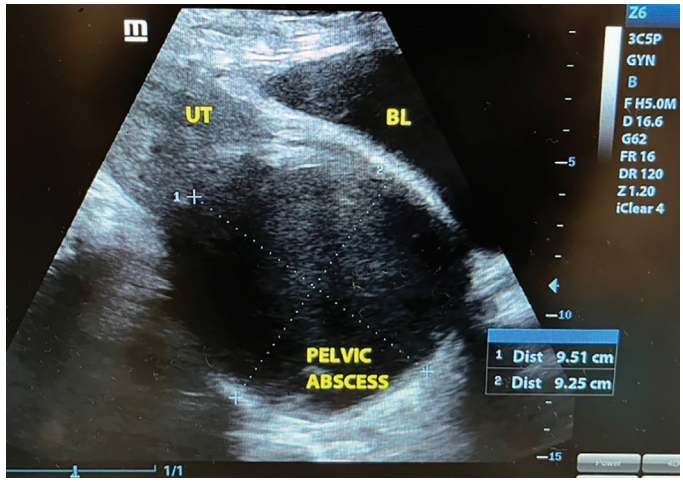

Figure 1. Ultrasonography image showing collection in leiomyoma

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Address for Correspondence: *Sunayna Lashkari*
e-mail: *lashkarisunayna@gmail.com* ***ORCID:*** *orcid.org/0009-0000-5837-9031*

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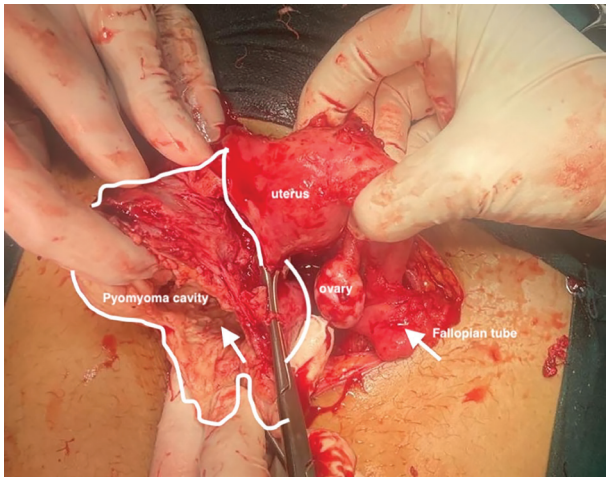

Figure 2. The pyomyoma dimensions are shown with a white outline over the posterior surface of the uterus, and its cavity is marked with a white arrow. The fallopian tube was edematous

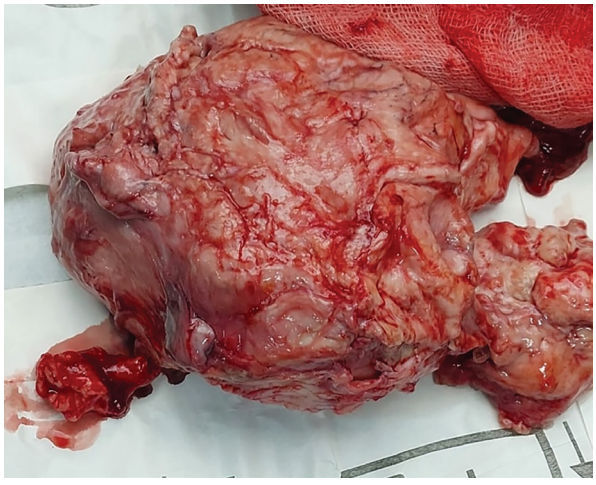

Figure 3. The necrosed fibroid with the pus inside was dissected from the uterus

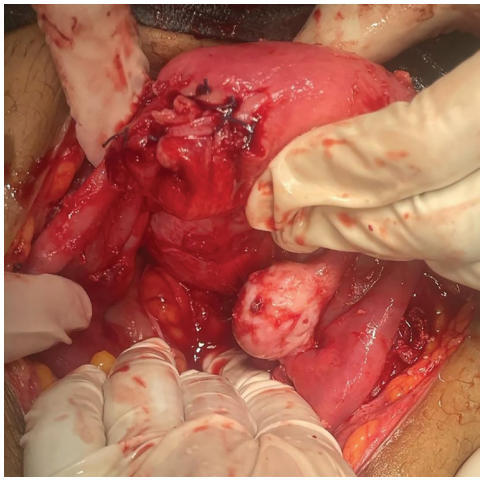

Figure 4. The repaired uterus after dissection of pyomyoma

preexisting infection, advanced maternal age, intravenous drug use, presence of intrauterine devices, co-morbid medical conditions, such as diabetes, hypertension and immunocompromise (3). Pre-labor rupture of membranes (1), ascending genital tract infection during the postpartum phase, and use of mechanical methods for the prevention of postpartum hemorrhage are also described as risk factors for leiomyomas. In the post-abortal phase, pyomyoma has been reported following spontaneous abortion (4) and uterine instrumentation.

To the best of our knowledge, this is the first case in which a patient developed pyomyoma following hysterotomy. This case emphasizes the importance of keeping pyomyoma as a differential diagnosis along with well-known causes, like tubo-ovarian abscess, pyometra, red degeneration of fibroid, endometritis, and septic abortion in a case of acute abdomen during pregnancy and/or the post-abortal and postpartum period.

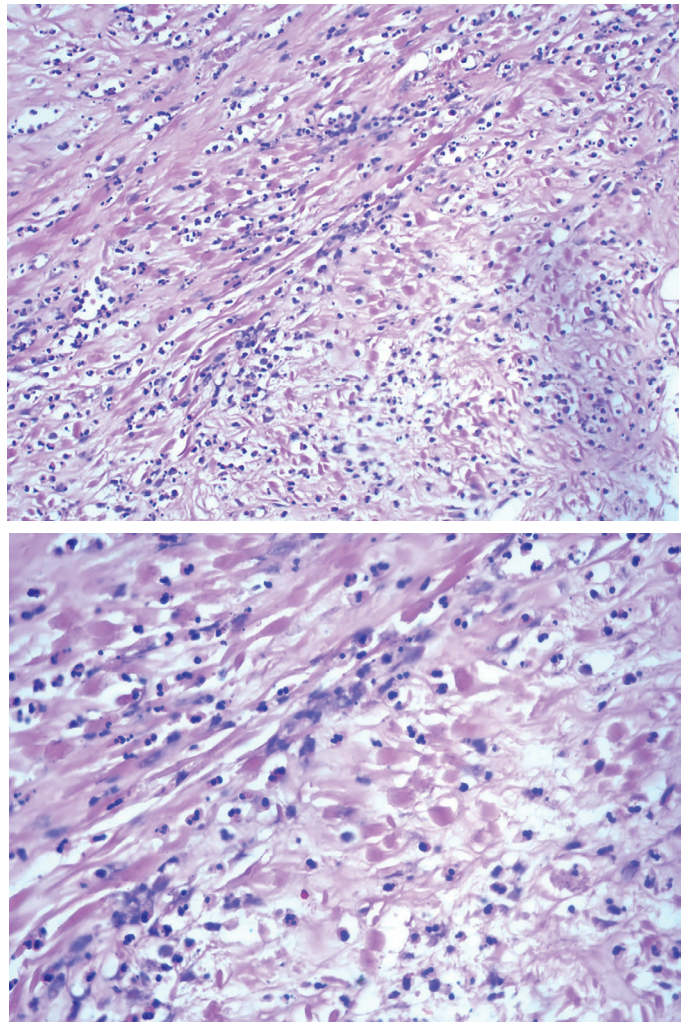

Figure 5. Histopathological images showing degenerated smooth muscle bundles with infiltration by neutrophils and areas of necrosis consistent with suppurative leiomyoma

Ethics

Informed Consent: *Written informed consent was obtained from the patient.*

Sunayna Lashkari, Avantika Gupta, Ayushi Sethi, Sahithi Kosgi

Department of Obstetrics and Gynaecology, All India Institute of Medical Sciences, Madhya Pradesh, India

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Robotic management of a ruptured rudimentary horn pregnancy

Anupama Bahadur[1], Udit Chauhan[2], Ayush Heda[1]

[1]Department of Obstetrics and Gynecology, All India Institute of Medical Sciences, Rishikesh, India
[2]Department of Radiodiagnosis, All India Institute of Medical Sciences, Rishikesh, India

Abstract

Unicornuate uterus results from incomplete Müllerian duct development and often includes a rudimentary horn. Pregnancy in such a rudimentary horn is rare. The video presents the robotic management of a 21-week, ruptured rudimentary horn pregnancy, emphasizing preoperative planning and surgical excision using a robotic platform, highlighting a novel minimally invasive technique for this rare obstetric emergency. A primigravida with a unicornuate uterus presented with a ruptured rudimentary horn pregnancy at 21 weeks and 4 days of gestation. Imaging confirmed fetal expulsion into the peritoneal cavity with moderate hemoperitoneum. The patient underwent right internal iliac artery embolization to minimize blood loss, followed by robotic excision of the ruptured horn, fetus, and placenta through posterior colpotomy. The procedure was completed using a multiport robotic setup with minimal intraoperative blood loss. The surgery was completed in 45 minutes with minimal complications. The patient had an uneventful postoperative recovery. Robotic-assisted surgery provides a safe, minimally invasive alternative to laparotomy for ruptured rudimentary horn pregnancies in well-selected patients. Meticulous planning, patient selection, and a multidisciplinary approach are key to success.

Keywords: Müllerian anomaly, obstetrics, robotic surgery

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Introduction

Unicornuate uterus results from incomplete formation and merging of Müllerian ducts and often includes a rudimentary horn, typically non-communicating in 75-90% of cases (1). Pregnancy in a rudimentary horn is rare, with an incidence of 1 in 76,000 to 1 in 140,000 (2). This video demonstrates the robotic management of a ruptured rudimentary horn pregnancy, showcasing a novel surgical approach for this condition.

Case report

A primigravida with a unicornuate uterus presented at 21 weeks and 4 days of gestation with a ruptured rudimentary horn pregnancy. On presentation, the patient was hemodynamically stable, with a tense abdomen, uterine size corresponding to 20 weeks of gestation, and the grooves sign was present. Bedside ultrasound revealed moderate pelvic fluid accumulation with a non-viable fetus in the peritoneal cavity, distinct from the uterus. Magnetic resonance imaging confirmed a bicornuate uterus with a ruptured right uterine horn, resulting in fetal expulsion into the peritoneal cavity and moderate hemoperitoneum.

Informed consent was obtained after discussing the planned approach, its benefits, risks, and alternative management options. Given the patient's stability (hemoglobin level 9.2 g/dL, disseminated intravascular coagulation score 2), and availability of a multidisciplinary team, including advanced interventional radiology and intensive care unit, right internal iliac artery embolization was performed to minimize

Address for Correspondence: *Anupama Bahadur*
e-mail: *anupama.bahadur@gmail.com* ***ORCID:*** *orcid.org/0000-0001-5582-0362*

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intraoperative blood loss, followed by robotic excision of the ruptured horn. The robotic-assisted procedure involved the removal of the horn, fetus, and placenta through posterior colpotomy (Figures 1-3). The port arrangement included a 12 mm supraumbilical camera port, two 8 mm robotic ports placed 10 cm lateral to the camera port on each side, and two 5 mm accessory ports for assistance, used for grasper and suction. The robotic instruments utilized during the procedure were a bipolar grasper and scissors.

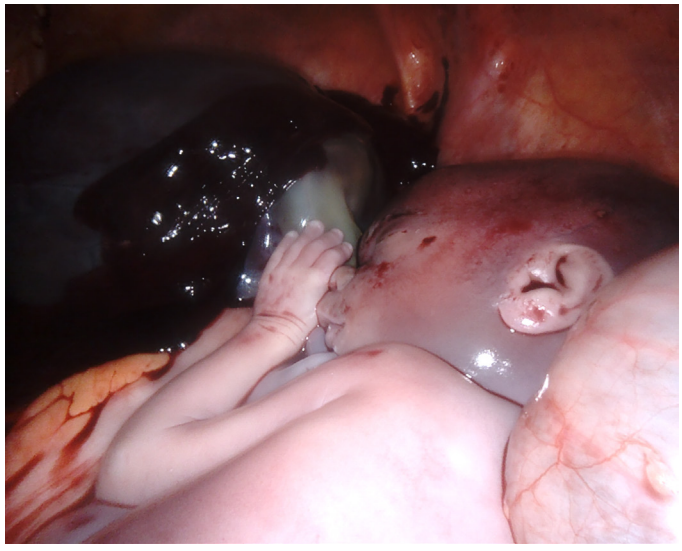

Figure 1. Fetus and placenta positioned within abdominal cavity, surrounded surrounded by blood clots

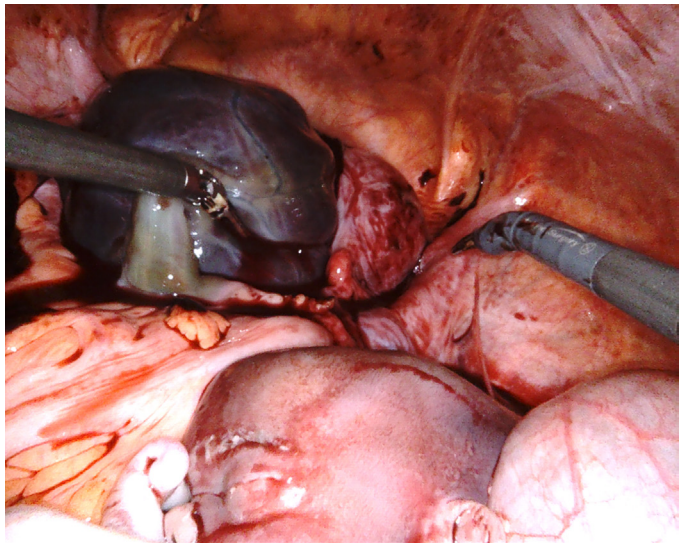

Figure 2. Extraction of the placenta from the ruptured rudimentary horn

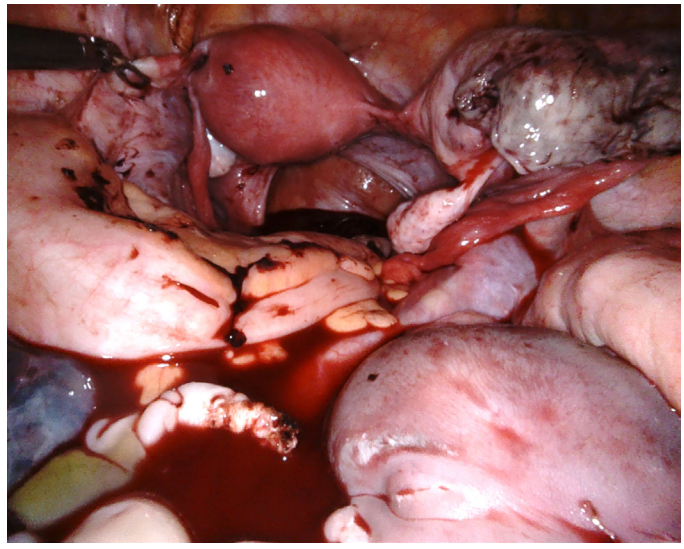

Figure 3. Uterus with normal left tube and ovary, and ruptured right rudimentary horn with intact right fallopian tube and ovary

The surgery was completed successfully within 45 minutes, including trocar placement, robotic docking, console time, and no comma needed minimal blood loss and no complications. Patient selection and available support services played a pivotal role in the success of this minimally invasive approach, based on hemodynamic stability, absence of significant coagulopathy, and comprehensive multidisciplinary support. The patient experienced an uneventful postoperative recovery, with minimal scarring and rapid return to normal activities. This case highlights the safety and efficacy of robotic-assisted surgery as an alternative to laparotomy for managing ruptured rudimentary horn pregnancies in well-selected patients in appropriate clinical settings.

Conclusion

Robotic-assisted surgery offers a safe and effective alternative to laparotomy for managing ruptured rudimentary horn pregnancies in carefully selected patients. This minimally invasive approach reduced blood loss, postoperative discomfort, and recovery time. Success depends on meticulous preoperative planning, appropriate patient selection, and availability of a well-coordinated multidisciplinary team.

Video 1.

http://dx.doi.org/10.4274/jtgga.galenos.2025.2025-3-1.video1

Ethics

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Conflict of Interest: *No conflict of interest is declared by the authors.*

Financial Disclosure: *The authors declared that this study received no financial support.*

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Erratum

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On the first page of the manuscript (page 286), it has been noted that a change in the author order has been requested. Accordingly, the necessary revision has been indicated within the text in bold. No changes have been made to the affiliation order.

The uncorrected version is as follows:
Rıza Madazlı[1], Hakan Erenel[1], Ayşegül Özel[1], Funda Öztunç[2]

The corrected version is as follows:
Hakan Erenel[1], Ayşegül Özel[1], Funda Öztunç[2], Rıza Madazlı[1]

CONGRESS CALENDER

INTERNATIONAL MEETINGS

(for detailed International Meeting please go website: https://www.emedevents.com/obstetrics-and-gynecology)

March 24-28, 2026	Society for Reproductive Investigation (SRI) 73rd Annual Scientific Meeting, San Juan, Puerto Rico
April 09-11, 2026	12th Congress of the Society of Endometriosis and Uterine Disorders (SEUD), Frankfurt, Germany
April 23-25, 2026	ASCCP 2026 Scientific Meeting, Orlando, USA
May 01-03, 2026	American College of Obstetricians and Gynecologists (ACOG) 2026 Annual Clinical and Scientific Meeting, Washington, USA
May 24-27, 2026	American Society for Reproductive Immunology (ASRI) Annual Meeting 2026, Lexington, USA
June 10-13, 2026	International Urogynecological Association (IUGA) 51st Annual Meeting, Rio De Janeiro, Brazil
June 17-29, 2026	The Society of Obstetricians and Gynecologists of Canada Annual Clinical Scientific Conference, Ottawa, Canada
July 05-08, 2026	European Society of Human Reproduction and Embryology (ESHRE) 42nd Annual Meeting, London, UK
September 06-09, 2026	36th ISUOG World Congess, Dubai, UAE
October 04-07, 2026	ESGE 35th Annual Congress, Krakow, Poland
October 24-28, 2026	American Society for Reproductive Medicine (ASRM) 82nd Annual Meeting, Baltimore, USA
November 12-14, 2026	The 34th World Congress on Controversies in Obstetrics Gynecology & Infertility (COGI), Athens, Greece
November 13-16, 2026	The 55th American Association of Gynecologic Laparoscopists (AAGL) Global Congress on Minimally Invasive Gynecologic Surgery (MIGS), Boston, USA

CONGRESS CALENDER

NATIONAL MEETINGS

(for detailed International Meeting please go website: https://www.kongreuzmani.com/2024)

April 22-26, 2026	2. Kadın Sağlığı Dernekleri Federasyonu Kongresi, Antalya, Türkiye
May 13-17, 2026	23. Ulusal Jinekoloji ve Obstetrik Kongresi, K.K.T.C.
May 15-19, 2026	5. Uluslararası Pelvik Taban ve Kozmetik Jinekoloji Kongresi, Antalya, Türkiye
May 15-19, 2026	3. Uluslararası Jinekoloji ve Obstetrik Derneği Kongresi, Antalya, Türkiye
September 30-October 04, 2026	8. Jinekoloji ve Obstetrikte Tartışmalı Konular Kongresi, Antalya, Türkiye

JTGGA CME/CPD CREDITING

Answer form for the article titled “Meta-analysis of changes in epithelial ovarian cancer incidence rates associated with salpingectomy: a comparison of 2022–2023 and earlier periods” within the scope of CME/CPD

1. **What is the primary medical reason for performing a salpingectomy mentioned in this paper?**
   a. To treat heart disease
   b. To prevent epithelial ovarian cancer (EOC)
   c. To improve eyesight
   d. To cure the common cold

2. **High-grade serous carcinoma, the most common EOC subtype, accounts for approximately what percentage of cases?**
   a. 25% to 30%
   b. 50% to 60%
   c. 70% to 75%
   d. 85% to 90%

3. **Recent research identifies which anatomical structure as the primary site of origin for many high-grade serous EOC cases?**
   a. The uterus
   b. The fallopian tubes
   c. The cervix
   d. The vaginal canal

4. **Which tool was used by the researchers to assess the methodological quality and risk-of-bias of the included studies?**
   a. The PRISMA Checklist
   b. The Newcastle-Ottawa Scale (NOS)
   c. The Cochrane Risk of Bias Tool
   d. The ACOG Scoring System

5. **The overall meta-analysis revealed that salpingectomy was associated with what percentage reduction in EOC incidence?**
   a. 45.5%
   b. 60.2%
   c. 77.7%
   d. 95.0%

6. **What is the medical term used in the paper for “removing the fallopian tubes”?**
   a. Hysterectomy
   b. Salpingectomy
   c. Appendectomy
   d. Biopsy

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1st Question			
A	B	C	D

2nd Question			
A	B	C	D

3rd Question			
A	B	C	D

4th Question			
A	B	C	D

5th Question			
A	B	C	D

6th Question			
A	B	C	D

People who answer these questions will receive *“2 TMA-CME/CPD credits”*

TMA-CME CREDITING BOARD ENQUIRY FORM

JTGGA MANUSCRIPT 2026/1

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Surname ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Signature ..... **The City You Work In** .....

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IMPORTANT NOTE: You may apply for Turkish Medical Association CME/CPD credits by answering the questions in the front page, filling in your personal information and sending this form to “Abdi İpekçi Cad. No: 2/7 34367 Nişantaşı, İstanbul” by post. This form should arrive to the above-mentioned address latest by 31 May, 2026.