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TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

## LETTER FROM THE PRESIDENT

Dear TJOD Family,

It is with great pleasure that I share with you the June issue of our scientific publication, the Turkish Journal of Obstetrics and Gynecology. I would like to extend my sincere gratitude to our editors, section editors, the reviewers who meticulously evaluated the submitted manuscripts, and, of course, to the national and international scientists who chose our journal to publish their valuable scientific work.

As you know, we held the 22nd National Turkish Congress of Gynecology and Obstetrics between May 14-18, 2025. With over 1,350 participants from across the country, this congress once again demonstrated its guiding role for today's obstetricians and gynecologists, thanks to its satellite symposia and up-to-date scientific sessions featuring both national and international experts.

I would also like to emphasize the significant momentum our journal has gained in recent years. Our readership continues to grow each year. In addition, our journal accepts submissions from numerous countries and publishes high-quality scientific articles following a thorough review process. It is worth noting that a significant portion of the submissions we receive are original research articles.

As we present our June issue to the esteemed gynecology and obstetrics community, I would like to underline that TJOD is not only a professional association but also the umbrella organization of a large and united family.

**Best Regards**

**Ismail Mete Itil, Prof. MD.**

**President of TJOD**

TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

## EDITORIAL

Dear Colleagues,

We are delighted to once again be with you through the June issue of the Turkish Journal of Obstetrics and Gynecology, the scientific publication of the Turkish Society of Gynecology and Obstetrics, as the second issue of 2025. In this issue, a considerable number of studies submitted by international scientists were evaluated.

After a rigorous selection process, ten articles with the highest scientific merit were included in the June issue. Eight of these are original research articles, while two are meta-analyses and review articles.

Additionally, the oral presentations delivered at the National Congress organized by the Turkish Gynecologic Oncology Foundation on April 5-6, 2025, in Ankara, have been published as a supplement to this issue.

We hope to meet you again in future issues of our scientific journal, which continues to advance with the principle of striving for excellence together.

**Ercan Yilmaz, Prof. MD.**
**Fatih Sendag, Prof. MD.**

*Clinical Investigation / Araştırma*

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Turk J Obstet Gynecol 2025;22(2):106-13

# Comparison of conventional karyotype analysis and CMA results with ultrasound findings in pregnancies with normal QF-PCR results

## *QF-PCR sonuçları normal olan gebeliklerde konvansiyonel karyotip analizi ve KMA sonuçlarının ultrason bulguları ile karşılaştırılması*

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### Abstract

**Objective:** In cases requiring fetal diagnostic testing, conventional karyotype analysis is initially preferred. However, quantitative fluorescent-polymerase chain reaction (QF-PCR) or fluorescent *in situ* hybridization methods are used alongside conventional karyotype analysis to obtain rapid results. If results cannot be obtained from conventional karyotype analysis, chromosomal microarray analysis (CMA) is a reasonable option in necessary cases. In this study, we analyzed the conventional karyotype and CMA results from pregnancies reported as having normal karyotypes by QF-PCR and assessed their correlation with ultrasound imaging results.

**Materials and Methods:** Between 2020 and 2023, pregnant women with fetal structural anomalies detected by ultrasound and magnetic resonance imaging at the Eskişehir City Hospital, Clinic of Perinatology were referred to our prenatal diagnosis center. In samples obtained using appropriate diagnostic methods, QR-PCR and conventional karyotype analysis were performed initially. Pregnancies with chromosomal anomalies detected by QF-PCR were excluded from the study. For pregnancies with normal karyotypes, CMA was applied.

**Results:** In 203 pregnancies with a normal karyotype result from QF-PCR, 202 (99.5%) were reported as normal in conventional karyotype analysis, while 1 (0.5%) case showed deletion of chromosome 7. Among the remaining pregnancies, CMA examination revealed abnormal karyotype results in 25 (12.3%) cases. A relationship was found only between ventriculomegaly detected by ultrasound and CMA results. The prevalence of ventriculomegaly was higher in those with CMA abnormalities (16%) compared to those with normal CMA (4.5%), and this difference was statistically significant (p=0.045).

**Conclusion:** The benefit of CMA analysis in detecting chromosomal anomalies such as copy number variations, especially in cases reported as having a normal karyotype by QF-PCR and karyotype analysis, is evident. To evaluate the relationship between ultrasound anomalies and CMA results, each community should assess its own results.

**Keywords:** Chromosomal microarray analysis, conventional karyotyping, fetal anomalies, quantitative fluorescent polymerase chain reaction

**PRECIS:** Comparison of conventional karyotype analysis and CMA results with ultrasound findings in pregnancies with normal QF-PCR results.

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## Öz

**Amaç:** Fetal tanı testi gerektiren durumlarda, başlangıçta konvansiyonel karyotip analizi tercih edilir. Ancak hızlı sonuç elde etmek için konvansiyonel karyotip analizinin yanı sıra kantitatif floresan-polimeraz zincir reaksiyonu (QF-PCR) veya floresan *in situ* hibridizasyon yöntemleri de kullanılmaktadır. Konvansiyonel karyotip analizinden sonuç alınamaması durumunda kromozomal mikroarray analizi (KMA) gerekli olgularda uygun bir seçenektir. Bu çalışmada, QF-PCR ile normal karyotiplere sahip olduğu bildirilen gebeliklerin konvansiyonel karyotip ve KMA sonuçlarını analiz ettik ve ultrason görüntüleme sonuçları ile korelasyonlarını değerlendirdik.

**Gereç ve Yöntemler:** 2020-2023 yılları arasında Eskişehir Şehir Hastanesi Perinatoloji Kliniği'nde ultrason ve manyetik rezonans görüntüleme ile fetal yapısal anomali saptanan gebeler prenatal tanı merkezimize yönlendirildi. Uygun tanı yöntemleri kullanılarak elde edilen örneklerde ilk olarak QR-PCR ve konvansiyonel karyotip analizi yapıldı. QF-PCR ile kromozomal anomali saptanan gebelikler çalışma dışı bırakıldı. Karyotipleri normal olan gebelere KMA analizi uygulandı.

**Bulgular:** QF-PCR ile normal karyotip sonucu elde edilen 203 gebeliğin 202'si (%99,5) konvansiyonel karyotip analizinde normal olarak rapor edilirken, 1 (%0,5) olguda delesyon 7 saptandı. Geri kalan gebelikler arasında, CMA incelemesi 25 (%12,3) olguda anormal karyotip sonuçları ortaya koymuştur. Sadece ultrason ile tespit edilen ventrikülomegali ile KMA sonuçları arasında bir ilişki bulunmuştur. Ventrikülomegali prevalansı KMA anormalliği olanlarda (%16) normal KMA olanlara (%4,5) kıyasla daha yüksekti ve bu fark istatistiksel olarak anlamlıydı (p=0,045).

**Sonuç:** Özellikle QF-PCR ve karyotip analizi ile normal karyotipe sahip olduğu bildirilen olgularda kopya sayısı varyasyonları gibi kromozomal anomalilerin tespit edilmesinde KMA analizi faydası belirgindir. Ultrason anomalileri ile KMA sonuçları arasındaki ilişkiyi değerlendirmek için her grup kendi sonuçlarını değerlendirmelidir.


**Anahtar Kelimeler:** Kromozomal mikroarray analizi, geleneksel karyotipleme, fetal anomaliler, kantitatif floresan polimeraz zincir reaksiyonu

## Introduction

Fetal structural anomalies detectable by ultrasound imaging (USI) occur at an average frequency of 3%[1]. When fetal structural anomalies are suspected or detected by USI, genetic examination is usually preferred. Chromosome abnormalities are found in 50-60% of miscarriages and stillbirths and in 1 in 150 live births[2]. These chromosome abnormalities primarily include trisomies, aneuploidies, chromosomal rearrangements, and monogenic disorders[3]. Although amniocentesis is the most commonly preferred method for genetic examination, techniques such as chorionic villus sampling (CVS) and cordocentesis are also used[4]. In recent years, non-invasive prenatal testing has become a part of our lives because it is free from both maternal and fetal complications[5]. However, although the accuracy rate is quite high, invasive diagnostic testing is still recommended for cases where chromosomal anomalies are detected[6]. The most common indications for these tests are advanced maternal age, increased risk in prenatal screening, and as previously mentioned, the detection of fetal structural anomalies by ultrasound[7].

Genetic examination technology first entered our lives in the 1960s with conventional cytogenetic analysis. Initially, G-banding technology was prominent. In the 1990s, fluorescent *in situ* hybridization and later quantitative fluorescent-polymerase chain reaction (QF-PCR) methods were introduced to overcome the limitations of fetal cell cultures, and to provide faster results by targeting selected chromosomes[8]. The QF-PCR method relies on the amplification of chromosome-specific DNA sequences (short tandem repeats) that vary in length. This technique, which is applied to chromosomes 13, 18, 21, and the sex chromosomes, provides results within 24-48 hours[9].

Subsequently, it has evolved into chromosomal microarray analysis (CMA). CMA, which detects imbalances in the kilobase range, easily demonstrates its superiority over standard karyotyping, which is limited to imbalances over 7-10 million bases[10]. Techniques for detecting submicroscopic pathogenic copy number variations (CNVs) are more successful in identifying imbalances of low mega base size (<50 kb)[11,12]. CNVs are often clinically insignificant, meaning that individuals with these CNVs are typically considered normal; however, this is not always the case. However, if they occur in a critical gene region or an important regulatory region, they may have functional effects. CMA is used not only during the prenatal period but also in the postnatal period[13]. In cases of structural anomalies that could not be diagnosed prenatally, as well as in cases with developmental delays and intellectual disability, the frequency of sub-chromosomal anomalies is found to be between 12% and 15%.

In this study, we compared the conventional karyotype analysis and CMA results, in fetuses with fetal structural anomalies detected by ultrasound in our tertiary care center, of pregnancies with normal karyotype reports from QF-PCR analysis.

Materials and Methods

This study was conducted after obtaining ethical approval from the Ethics Committee of Eskişehir City Hospital (no: ESH/GOEK 2024/80, date: 14.03.2024) and after informing all parents verbally and obtaining their written consent. Between 2020 and 2023, pregnant women with fetal structural anomalies detected by our most frequently used prenatal imaging methods, ultrasound and magnetic resonance imaging, were referred to our prenatal diagnostic center. Ultrasound findings were recorded by the same perinatologist (ZB) using a Voluson E8 (General Electric Company, Istanbul, Türkiye). Fetal samples were collected through chorionic villus sampling, amniocentesis, and cordocentesis. The choice of method was determined, in consultation with the parents, based on the gestational age. Verbal and written consent was obtained from all families. The invasive procedures were performed by the same perinatologist (ZB), and no complications were observed. Multiple pregnancies, cases with inadequate material in invasive

diagnostic tests, cases with placental mosaicism, and cases with positive anomalies in QF-PCR were excluded from the study.

### Karyotype Analysis

Two hundred and eighty-two pregnant women who met the study criteria, were included in the study. QF-PCR analysis was performed for X, Y, sex chromosome anomalies and for chromosomes numbered 13, 18, and 21. Cases with normal karyotypes were removed from consideration, resulting in a final sample of 203 study cases. Following this, conventional karyotype analysis was performed using standard G-banding technology.

### CMA Analysis

Array analysis was performed using the Affymetrix Cytoscan Optima Suite method with a 315k resolution, utilizing the GRCh37 reference genome. Duplications greater than 200.000 and deletions greater than 400.000 were considered significant for prenatal diagnosis. According to ACMG Guidelines, smaller deletions and duplications were considered significant in the presence of clinical results.

### Statistical Analysis

SPSS 25.0 (IBM Corporation, Armonk, New York, United States) was used for variable analysis. The normality of the data was assessed using the Shapiro-Wilk test. For comparing quantitative variables between two groups, Mann-Whitney U tests with Monte Carlo simulation results were used. Categorical variables were compared using the Fisher's exact test with Monte Carlo simulation techniques. The odds ratio, along with 95% confidence intervals, was employed to quantify the likelihood of individuals with a risk factor relative to those without it. Quantitative variables are expressed as mean (standard deviation) and median (minimum/maximum) in the tables, while categorical variables are shown as n (%). Variables were examined at a 95% confidence level, and a p-value of less than 0.05 was considered significant.

## Results

Two hundred and three pregnancies that met the inclusion criteria and had normal karyotype results from QF-PCR, were included in the study for conventional karyotype analysis and CMA results. The parity, gestational age, risk level values from the screening test, the number of findings in the ultrasound, and the procedures performed for the included pregnancies are listed in Table 1. The average participant has one previous pregnancy, as indicated by a mean of 1 and a standard deviation of 1.03. The average gestation period among participants is approximately 20 weeks, with a median gestation week of 21. This median indicates that half of the participants are at or beyond the 21-week mark, highlighting a distribution that encompasses both early and later stages of pregnancy, with gestation weeks ranging from 12 to 31.

The data further elucidate the prevalence of specific prenatal diagnostic procedures. A significant majority of the women (78.3%) underwent amniocentesis, a widely accepted prenatal diagnostic intervention. In contrast, cordocentesis was performed on 9.9% of participants, while CVS was conducted on 11.8%. These figures underscore the predominant reliance on amniocentesis compared to the other diagnostic options, reflecting its established role in prenatal care.

Moreover, the analysis of USI findings reveals that 21.2% of participants reported no abnormalities, while the majority reported a single finding, which was noted in 39.4% of cases. As the number of findings increased, the frequency of occurrences decreased, indicating that cases involving multiple ultrasound findings are less common. Specifically, only 2% of women exhibited five findings, and 1% displayed six findings.

The QF-PCR result indicated a normal karyotype. The conventional karyotype and CMA analyses for the 203 pregnant women included in the study are presented in Table

**Table 1.** Demographic characteristics of pregnant women

| | Mean (standard deviation) | Median (minimum/maximum) |
|---|---|---|
| **Parity (n)** | 1 (1.03) | 1 (0/8) |
| **Gestation week (week)** | 19.93 (3.75) | 21 (12/31) |
| **Risk in screening test (10000)** | 32.76 (59.64) | 5.47 (1/476.19) |
| **Process** | | |
| **Amniocenteses** | 159 (78.3) | |
| **Cordocentesis** | 20 (9.9) | |
| **CVS** | 24 (11.8) | |
| **Number of USI findings (n)** | | |
| 0 | 43 (21.2) | |
| 1 | 80 (39.4) | |
| 2 | 44 (21.7) | |
| 3 | 23 (11.3) | |
| 4 | 7 (3.4) | |
| 5 | 4 (2.0) | |
| 6 | 2 (1.0) | |

USI: Ultrasound imaging, CVS: Chorionic villus sampling

**Table 2.** Distribution of chromosomal microarray analysis and conventional karyotype results of pregnant women

| | n (%) |
|---|---|
| **Microarray (anormal)** | 25 (12.3) |
| **Karyotype** | |
| Normal | 202 (99.5) |
| Deletion 7 | 1 (0.5) |

2. The distribution of USI findings of pregnant women who underwent genetic analysis and had a normal karyotype as a result of QF-PCR is shown in Table 3. The most commonly observed abnormalities, including an increase in nuchal fold measurements and hyperechogenic bowel, were reported in 25 cases (12.3%). Similarly, an intracardiac hyperechogenic focus was noted in 24 cases (11.8%).

Among the other notable anomalies, pyelectasis and muscular ventricular septal defect were each identified in 20 cases (9.9%). The findings also included ventriculomegaly, observed in 12 cases (5.9%). Additional notable conditions consisted of aberrant right subclavian artery, identified in 10 cases (4.9%), and nasal bone hypoplasia, found in 9 cases (4.4).

As the analysis of ultrasound findings continues, microcephaly and cervical cystic hygromas were reported in 7 instances each (3.4%). Moreover, a range of abnormalities, such as clenched hand, forearm dysplasia, and early-onset intrauterine growth restriction, accounted for 6 cases (3%). These findings highlight the varied nature of potential anomalies detectable during prenatal imaging.

Among the less frequently identified abnormalities, more complex conditions emerged, including mega cisterna magna and posterior urethral valve, each found in 5 cases (2.5%). Additionally, a remarkable array of anomalies was documented in single instances (0.5%), such as syndactyly and interrupted aortic arch, emphasizing the diversity of developmental issues that may be encountered during prenatal assessments.

The findings presented in this table highlight the complexity and range of abnormalities that can be detected through USI in a population that ultimately demonstrates normal chromosomal results.

No statistically significant difference was found in the comparison of the demographic characteristics of those who were pregnant, based on the CMA results (p>0.05) (Table 4).

Among the ultrasound findings and CMA results in pregnant women, only ventriculomegaly was found to be associated with the outcomes. The incidence of ventriculomegaly was significantly higher in those with abnormal CMA (16%) compared to those with normal CMA (4.5%) (p=0.045). Pregnant women with abnormal CMA had a fourfold (1.1-14.6 times higher) rate of ventriculomegaly compared to those with normal CMA (Table 5).

**Table 3.** Distribution of USI findings of pregnant women, who underwent genetic analysis and had a normal karyotype as a result of QF-PCR

| | n (%) |
|---|---|
| Nuchal fold increase, hyperechogenic bowel | 25 (12.3) |
| Intracardiac hyperechogenic focus | 24 (11.8) |
| Pyelectasis | 20 (9.9) |
| Muscular VSD | 20 (9.9) |
| Ventriculomegaly | 12 (5.9) |
| Aberrant right subclavian artery | 10 (4.9) |
| Nasal bone hypoplasia | 9 (4.4) |
| Microcephaly | 7 (3.4) |
| CPC | 7 (3.4) |
| Clenched Hand, forearm dysplasia, early onset IUGR | 6 (3) |
| Mega Cisterna Magna, CSP width, posterior urethral valve, NT increase | 5 (2.5) |
| hypoplasia of the cerebellum, cleft palate and lip, pericardial effusion, toxoplasma, rhizomelia | 4 (2) |
| Pes Equinovarus, partial corpus callosum agenesis, aortic coarctation | 3 (1.5) |
| Placental calcification, double outlet right ventricle, hypoplasia of the thorax, micromelia, ambiguous genitalia, FL shortness, stomach width, right aortic arch, single artery single vein, AVSD, encephalocele, liver calcification, megacystis, corpus callosum agenesis | 2 (1) |
| Syndactyly, double collecting system, ınterrupted aortic arch, hypoplastic stomach, cell-free DNA Anomaly, myopathy carrier, hyperechogenic kidney, gallbladder agenesis, rocker bottom feet, costa hypoplasia, acrocephaly, double renal artery, CMV, corrected TGA, ductus venosus agenesis, hypoplastic kidney, left superior vena cava, thymus hypoplasia, SMA carrier, CPAM, gastroschisis, isomerism, holoprosencephaly, microphthalmia, flat face, Cantrell, PKU carrier, abdominal ascites, blake pouch cyst, polycystic kidney, micrognathia, leg dysplasis, hypotonia, low ear, persistent right umbilical vein, renal agenesis, arachnoid cyst, clinodactyly, vermis hypoplasia, diaphragmatic hernia | 1 (0.5) |

USI: Ultrasound imaging, QF-PCR: Quantitative fluorescent-polymerase chain reaction, VSD: Ventricular septal defect, IUGR: Intrauterine growth restriction, NT: Nuchal translucency, CMV: Cytomegalovirus, AVSD: Atrioventricular septal defect, TGA: Transposition of the great arteries, SMA: Spinal musculer atrophy, CPAM: Cystic pulmonary airway malformation, PKU: Phenylketonuria

**Table 4.** Comparison of demographic characteristics of pregnant women according to CMA results

| | Microarray (normal) (n=178) Median (minimum/maximum) | Microarray (anormal) (n=25) Median (minimum/maximum) | p |
|---|---|---|---|
| **Parity (n)** | 1 (0/8) | 1 (0/2) | 0.450[u] |
| **Gestation week (week)** | 21 (12/31) | 21 (12/26) | 0.340[u] |
| **Risk in screening test (10000)** | 3.56 (1/263.16) | 10.33 (1/476.19) | 0.073[u] |
| **Invasive test indication (n)** | 1 (0/2) | 1 (0/2) | 0.090[u] |
| | **n (%)** | **n (%)** | |
| **Process** | | | 0.367[f] |
| Amniocentesis | 141 (79.2) | 18 (72.0) | |
| Cordocentesis | 18 (10.1) | 2 (8.0) | |
| CVS | 19 (10.7) | 5 (20.0) | |
| **Number of USI findings (n)** | | | 0.820[f] |
| 0 | 38 (21.3) | 5 (20.0) | |
| 1 | 71 (39.9) | 9 (36.0) | |
| 2 | 39 (21.9) | 5 (20.0) | |
| 3 | 18 (10.1) | 5 (20.0) | |
| 4 | 6 (3.4) | 1 (4.0) | |
| 5 | 4 (2.2) | 0 (0.0) | |
| 6 | 2 (1.1) | 0 (0.0) | |

[f]: Fisher exact test (Monte Carlo), [u]: Mann-Whitney U test (Monte Carlo), CMA: Chromosomal microarray analysis, USI: Ultrasound imaging, CVS: Chorionic villus sampling

**Table 5.** Comparison of USI findings with CMA results

| | Microarray (normal) (n=178) n (%) | Microarray (anormal) (n=25) n (%) | Odds ratio (95% confidence interval) | p |
|---|---|---|---|---|
| **Microcephaly** | 5 (2.8) | 2 (8) | | 0.208 |
| **Rhizomelic** | 2 (1.1) | 2 (8) | | 0.075 |
| **CPC** | 7 (3.9) | 0 (0) | | 0.600 |
| **Nuchal fold increase** | 21 (11.8) | 4 (16) | | 0.521 |
| **Pyelectasis** | 18 (10.1) | 2 (8) | | 0.999 |
| **Ventriculomegaly** | 8 (4.5) | 4 (16) | 4 (1.1-14.6) | 0.045 |
| **Nasal bone hypoplasia** | 9 (5.1) | 0 (0) | | 0.605 |
| **Early onset IUGR** | 5 (2.8) | 1 (4) | | 0.55 |
| **Hyperechogenic bowel** | 24 (13.5) | 1 (4) | | 0.325 |
| **Cerebellum hypoplasia** | 4 (2.2) | 0 (0) | | 0.999 |
| **Intracardiac hyperechogenic focus** | 21 (11.8) | 3 (12) | | 0.999 |
| **Muscular VSD** | 17 (9.6) | 3 (12) | | 0.719 |
| **Toxoplasma** | 4 (2.2) | 0 (0) | | 0.999 |
| **Mega cisterna magna** | 4 (2.2) | 1 (4) | | 0.486 |

**Table 5.** Continued

| | Microarray (normal) (n=178) n (%) | Microarray (anormal) (n=25) n (%) | Odds ratio (95% confidence interval) | p |
|---|---|---|---|---|
| **Cleft palate and lip** | 4 (2.2) | 0 (0) | | 0.999 |
| **CSP width** | 4 (2.2) | 1 (4) | | 0.485 |
| **Posterior urethral valve** | 4 (2.2) | 1 (4) | | 0.485 |
| **Pericardial effusion** | 3 (1.7) | 1 (4) | | 0.411 |
| **Clenched hand** | 6 (3.4) | 0 (0) | | 0.999 |
| **Forearm dysplasia** | 6 (3.4) | 0 (0) | | 0.999 |
| **NT increase** | 5 (2.8) | 0 (0) | | 0.999 |

Fisher's exact test (Monte Carlo), USI: Ultrasound imaging, CMA: Chromosomal microarray analysis, IUGR: Intrauterine growth restriction, NT: Nuchal translucency, VSD: Ventricular septal defect, CSP: Cavum septum pellusidi, CPC: Choroid plexus cyst

## Discussion

Although the QF-PCR method is advantageous in terms of cost and time, further evaluation is recommended for cases with normal karyotype results. Bartels et al.[14] assessed the outcomes of QF-PCR and amniocentesis in 528 cases over a 5-year period. QF-PCR identified genetic anomalies in 32% of the cases, including trisomy 21, 18, 13, and other conditions. Standard karyotype analysis revealed anomalies in 36.2% of cases. In 21 instances, different results were observed, with DiGeorge syndrome being the most common anomaly, occurring in 7 cases. Liao et al.[15] analyzed amniocentesis materials and found a genetic anomaly rate of 2.5%. Out of 211 cases with genetic abnormalities, QF-PCR failed to diagnose 43. The overall residual risk was calculated to be 0.1%. Comas et al.[16] identified 110 abnormal karyotypes, representing a rate of 2.8%, and found that 27% of these could not be diagnosed by QF-PCR. In their study, the overall residual risk was 0.75%. According to these findings, QF-PCR is a cost-effective and acceptable method for selected cases. Papoulidis et al.[17] reviewed their study results, which included a larger number of cases compared to other studies. Chromosomal abnormalities were detected in 2.37% of cases. Out of 320 cases, 70 could not be diagnosed by QF-PCR. Approximately half of these were already at high risk. When evaluating USI findings and genetic history, 13 cases were identified as missed by QF-PCR, which corresponds to 0.1%. Given this information, although "selective dual testing" is recommended, the 0.1% error rate indicates that alternative options should be considered. In our study, abnormal karyotype results were found in only 1 (0.5%) of the cases where QF-PCR results were reported as normal. This rate is lower than that reported in previous studies.

CMA is particularly successful in analyses involving CNVs that conventional karyotype analysis fails to detect. In a study by Wapner et al.[18], which examined more than 4,000 samples from 29 centers, cases reported as normal karyotypes by conventional methods were evaluated by CMA. Accordingly, 6% of small deletions and duplications (CNVs) were detected. The study found that CMA was useful for identifying aneuploidies and unbalanced rearrangements. However, it may be insufficient for detecting balanced translocations and triploidies. In the review prepared by Callaway et al.[19], CMA was performed on pregnant women, who had normal results from conventional karyotype testing. The CNV rate ranged from 0.8% to 5.5%, with a mean rate of 2.4%. The rate of abnormal fetal USI results in these pregnant women varied from 6.0% to 11.1%, with an average of 6.5%. The review also included an analysis of pregnant women with abnormal fetal USI results, finding CNVs in 7% of fetuses with abnormal USI. Based on these results, CMA was recommended as a primary test. In our study, the CNV detection rate was 12.3%, which is consistent with that reported in the literature.

Approximately half of pregnancy losses have been associated with genetic anomalies[20]. In a meta-analysis of 9 studies by Dhillon et al.[21], it was found that CMA and conventional karyotype analyses produced the same results in 86% of cases, while CMA provided additional information in 13% of cases. The rate of variants of unknown significance (VUS) was 2%. Interestingly, karyotype analysis detected additional anomalies that CMA missed in 3% of cases. Another meta-analysis by Pauta et al.[22] focused on cases of early pregnancy loss. In addition to karyotype analysis, CMA revealed pathological CNVs in 2% of cases and VUS in 4%. The most common CNVs identified were deletions at 22q11.21 and 1p36.33. Reddy et al.[23] evaluated pregnant women who experienced stillbirth. Compared to conventional karyotyping, CMA demonstrated a 41.9% relative increase in the detection of genetic abnormalities. For stillbirths with fetal anomalies, this increase was approximately 53%. In the meta-analysis by Martinez-Portilla et al.[24], the success rate of conventional karyotyping was 75%, while CMA achieved a success rate of 90%. In CMA, CNV was detected in 4% and VUS

in 8%. In our study, genetic materials of fetuses that resulted in miscarriage and stillbirth were excluded.

First and second trimester screenings with fetal USI identify pregnant women who require detailed USI and targeted USI fetal chromosome analysis. Grande et al.[25] analyzed the CMA results of pregnant women with NT ≥3.5 mm, and a normal karyotype. CNVs were detected at a rate of 5%. The most common anomalies included deletions and duplications at 22q11.2 and deletions at 10q26.1, q26.3. In our study, there was no statistically significant difference in NT measurements between cases with normal and abnormal CMA results. In a systematic review by de Wit et al.[26] evaluating 18 studies, the presence of CNV ranging from 3.1-7.9%, was found in the presence of one anatomical system anomaly detected by USI. In the presence of multiple anomalies, this rate was found to be 9.1%. In our study, there was no statistically significant difference between the frequencies of USI anomalies and the frequencies of CMA results. This is not aligned with findings reported in the general literature. We believe this situation occurred because our study included fewer patients compared to the large case series we discussed.

Hui et al.[27] compared single system anomalies and non-specific results. Thus, cardiac anomalies were identified as present in the group with the greatest risk. Among the non-specific results, fetal growth retardation was present in the group with the highest rate of CNV. In our study, it was very difficult to evaluate growth retardation because the USI weeks were usually between weeks 18 and 22. Shaffer et al.[28] conducted a more detailed study on USI anomalies. CNV was detected in 5.6% for a single USI anomaly and 9.5% for multiple USI anomalies. What is important is the subanalysis of USI anomalies. In the analysis performed without considering the association with another anomaly, isolated left heart hypoplasia was found in 16.2%, posterior fossa anomaly in 14.6%, and skeletal system anomaly in 10.7%. In our study, only ventriculomegaly was statistically different between the normal and abnormal groups in CMA analysis. We think that the reason for the different results in our study may be due to the small number of patients and/or racial factors.

The correlation observed between ultrasound findings and CMA results is particularly insightful. The significant association of ventriculomegaly with abnormal CMA results raises important clinical considerations. The predominance of certain anomalies, such as increases in nuchal fold measurement, aligns with existing literature emphasizing the importance of these markers in prenatal screenings. However, the identification of more rare conditions like interrupted aortic arch and syndactyly highlights the necessity for thorough ultrasound evaluations to ensure no significant anomaly is overlooked, even in populations where normal chromosomal results are expected. This demonstrates the critical role of advanced imaging in conjunction with genetic testing to improve prenatal counselling and management strategies.

### Study Limitation

One limitation of our study is that it was conducted at a single center, involving the same ethnicity, and involving a small number of patients.

## Conclusion

CMA is especially useful for detecting chromosomal abnormalities when QF-PCR and karyotype analysis report normal results. To understand the relationship between USI abnormalities and CMA results, each society should analyze its results based on its own socio-demographic characteristics.

### Ethics

**Ethics Committee Approval:** This study was conducted after obtaining ethical approval from the Ethics Committee of Eskişehir City Hospital (no: ESH/GOEK 2024/80, date: 14.03.2024).

**Informed Consent:** Verbal and written consent was obtained from all families.

### Footnotes

### Authorship Contributions

Surgical and Medical Practices: Z.B., M.K., G.Ş., E.A., E.E.G., S.A., Concept: Z.B., Design: M.K., E.A., Data Collection or Processing: M.K., E.A., Analysis or Interpretation: G.Ş., E.E.G., S.A., Literature Search: G.Ş., Writing: Z.B., M.K.

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

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# Is there a correlation between the severity of symptoms and vitamin D levels in pregnancy with hyperemesis gravidarum?

## *Hiperemezis gravidarumlu gebelerde semptomların şiddeti ile serum D vitamini düzeyleri arasında korelasyon var mı?*

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### Abstract

**Objective:** This study aimed to investigate the relationship between serum vitamin D levels and the severity of symptoms in individuals with hyperemesis gravidarum (HG).

**Materials and Methods:** A cohort of eighty patients exhibiting vomiting three or more times daily, positive ketones on complete urinalysis, and oral feeding difficulties were recruited. Symptom severity was assessed using the 8-question Rhodes index, categorizing patients into non-symptomatic, mild, moderate, and severe groups. Serum vitamin D levels were measured using venous blood samples; a deficiency was defined as less than 10 ng/mL, an insufficiency as 10-20 ng/mL, and a normal level as more than 20 ng/mL.

**Results:** The distribution of symptom severity revealed 14 (17.5%) with mild, 38 (47.5%) with moderate, and 28 (35%) with severe symptoms. Groups showed no significant differences in demographic or obstetric characteristics except for ketone positivity rates ($p<0.05$). There was a significant difference in vitamin D levels between the severity groups: mild symptoms (32.12±4.02 ng/mL), moderate symptoms (19.98±6.37 ng/mL), and severe symptoms (8.11±3.06 ng/mL) ($p<0.001$). Vitamin D and the Rhodes index mean score showed a significant negative relationship ($r=-0.844$, $p=0.001$). With 96.4% sensitivity and 89.5% specificity, receiver operating characteristic analysis showed that symptom intensity rose when blood vitamin D levels were less than 11.54 ng/mL.

**Conclusion:** These results highlight a negative relationship between the severity of HG symptoms and serum vitamin D levels. Screening pregnant women with nausea, vomiting, and severe symptoms for serum vitamin D deficiency is recommended. Appropriate pre-pregnancy treatment should be initiated for those deficient or insufficient in serum vitamin D to potentially alleviate HG symptom severity and frequency.

**Keywords:** Pregnancy, vitamin D, Rhodes index, vomiting

**PRECIS:** Using a 40 points questionnarie, we observed a negative correlation between the severity of HG and serum vitamin D levels.

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## Öz

**Amaç:** Bu çalışmanın amacı hiperemezis gravidarum (HG) hastalarının semptomlarının şiddeti ile serum D vitamini düzeyleri arasındaki ilişkiyi ortaya koymaktır.

**Gereç ve Yöntemler:** Günde 3 veya daha fazla sayıda kusma şikeyeti olan, yapılan tam idrar tetkikinde keton pozitifliği tespit edilen ve oral beslenme zorluğu bulunan 80 hasta çalışmaya dahil edildi. Hastalara, semptomlarının şiddetini belirlemek için 8 soruluk Rhode'nin indeks skorlama anketi uygulandı. Anket sonucunda 8 puanın altında olan hastalar semptomatik olmayan grup olarak görülürken, 9-18 puan arasında olanlar hafif, 19-32 puan arasında olanlar orta, 33-40 puan arasında olanlar şiddetli semptomatik grup olarak belirlendi. Tüm hastalardan serum D vitamini seviyesini belirlemek için 5 mL venöz kan örneği alındı. Serum D vitamin düzeyi <10 ng/mL olan hastalar eksiklik, 10-20 ng/mL arasında olanlar yetersizlik ve >20 ng/mL olanlar normal seviyede D vitaminine sahip grup olarak sınıflandırıldı.

**Bulgular:** Rhode'nin indeks skorlamasına göre, çalışmaya dahil edilen 80 hastanın 14'ü (%17,5) hafif, 38'i orta (%47,5), 28'i (%35) ağır semptomatik gruba dahil oldu. Gruplar arasında yaş, gebelik, doğum, abortus, yaşayan çocuk sayıları, gebelik haftaları ve vücut kitle indeksleri bakımından anlamlı farklılık yoktu. Hafif, orta ve ağır derecede semptomatik gruplarda keton pozitiflik oranları bakımından anlamlı farklılık saptandı (p<0,05). Gruplar arasında D vitamini düzeyleri bakımından anlamlı farklılık mevcuttu. Hafif semptomatik grupta D vitamini seviyesi 32,12±4,02 ng/mL, orta düzeyde semptomatik grupta 19,98±6,37 ng/mL ve ağır semptomatik grupta 8,11±3,06 ng/mL idi (p<0,001). Rhode'nin indeks skoru ile D vitamini arasında negatif yönde çok güçlü bir korelasyon saptandı (r=-0,844, p=0,001). Alıcı çalışma karakteristiği analizinde, serum D vitamini seviyesi <11,54 ng/mL olduğunda %96,4 duyarlılık ve %89,5 özgüllük ile, bulantı-kusma şiddet ve sıklığının arttığı izlendi.

**Sonuç:** Serum D vitamin seviyeleri ile HG semptomlarının şiddeti arasında negatif yönde güçlü bir ilişki vardır. Bu sonuçlarla,bulantı-kusması olup, ciddi semptom yaşayan gebelerde serum D vitamin düzeyinin araştırılmasının ve/veya gebelik öncesinden başlayarak, serum D vitamin düzeyleri bakımından eksiklik ve/veya yetersizlik seviyesinde değeri olan gebelerde uygun ilaçlarla tedavinin, HG semptomlarının şiddetini ve sıklığını azaltmada uygun bir yöntem olacağını düşünmekteyiz.


**Anahtar Kelimeler:** Gebelik, D vitamini, Rhodes indeks, kusma

## Introduction

Hyperemesis gravidarum (HG) is a significant medical condition affecting approximately 1% of pregnant individuals, resulting in significant morbidity for both the mother and the fetus[1]. The etiology of HG remains unclear, with numerous theories having been proposed. Among these theories are psychological factors, hormonal changes, gastrointestinal dysmotility, and immunological dysregulation[2,3]. It is noteworthy that up to 80% of pregnant women experience some degree of nausea and vomiting; however, cases involving long-duration complaints are classified as HG. This condition manifests in 0.5-2% of all pregnancies[4]. The symptoms generally appear on the 5$^{th}$ or 6$^{th}$ day of pregnancy. It begins in the first weeks of pregnancy, reaches its peak in the 9th week, and continues from the 16$^{th}$ to the 20$^{th}$ week. It has been observed that the symptoms begin to disappear after the first week of pregnancy[5]. The severity of symptoms can range from mild to severe. While some pregnant women experience weight loss due to this condition, it can also lead to fluid, electrolyte, and acid-base imbalances, which may result in nutritional deficiency and necessitate hospitalization[6,7].

The body uses vitamin D as a well-known immunomodulator and anti-inflammatory. Numerous disorders affecting the reproductive system have been linked to vitamin D deficiency[8,9]. Sugito et al.[10] discovered that pregnant women with HG had higher blood levels of cell-free DNA. This disorder is thought to be caused by the mother's immune system overactivity and trophoblast destruction. Women with HG have been shown to have an active immune system. The etiopathogenesis of HG is believed to be significantly influenced by vitamin D, and deficiencies in this vitamin may contribute to immune regulatory issues and explain the immunological hypothesis of HG[11]. Teenagers sometimes suffer from vitamin D insufficiency. Low plasma concentrations are found in around 40% of African American women and 4% of non-Hispanic Caucasian women. Even in developed nations, pregnant women's vitamin D levels are concerning[12]. Overall, enhanced newborn calcium utilization results from vitamin D treatment in at-risk groups[13]. Initially, the number of vomiting episodes per day and the intensity of nausea were used to define the symptomatology of nausea and vomiting of pregnancy (NVP). However, Rhodes' description of the revolution in quantifying NVP symptomatology included the measurement of nausea, vomiting, and retching characteristics, and validated these in addition to the length and number of attacks[14]. Although the Rhodes score was created for nausea and vomiting secondary to cancer chemotherapy, it has been adapted for NVP in recent years[15,16].

The present study aimed to group pregnant women diagnosed with HG according to the severity of symptoms, and to reveal whether there is a difference in vitamin D levels between these groups. Thus, it was also aimed to initiate crucial studies on whether vitamin D can play an active role in both the etiology and treatment of severe nausea and vomiting, which is an important problem for pregnant women

## Materials and Methods

Patients with nausea and vomiting complaints, three or more vomiting complaints per day, a full urine test showing ketone positivity, and difficulty with oral feeding, who applied to Gaziantep University Faculty of Medicine, Gynecology and Obstetrics Polyclinic between August 1, 2020, and February 1, 2021, were included in the study. The research involved eighty patients. The Faculty of Medicine Gaziantep University Clinical Research Ethics Committee granted approval for this

study, which was carried out in compliance with the Helsinki Declaration's criteria (approval number: 241/2020; date: 16.07.2020).

The following information was recorded for the research participants: age, body mass index (BMI), number of living children, weeks of gestation and ketone positive readings in a complete urine sample.

The study excluded pregnant women with trophoblastic illness, thyroid and gastrointestinal disorders before or during pregnancy, infections, multiple pregnancies, and drug use for any reason. After the patients were provided with the information they needed for the study, they completed the informed consent form.

The patients' serum vitamin D levels were measured using a 5 mL venous blood sample. Within two hours of withdrawal, the samples were centrifuged and kept at -20 °C until analysis. Serum vitamin D levels below 10 ng/mL were considered deficient, those between 10 and 20 ng/mL were considered insufficient, and those over 20 ng/mL were considered normal.

### Rhodes Index

Each patient's level of nausea and vomiting was assessed using an 8-item Rhodes index. The Rhodes index's scoring methodology questioned patients if they had suffered from any pain in the previous 12 hours, and if they experienced symptoms such as nausea, vomiting, and retching, and how severe such symptoms were. The Rhodes index is a scoring system that allows the evaluation of both objective and subjective aspects of nausea and vomiting. In scoring, 1 point indicates the presence of minimal or no symptoms, while 5 points represent the most severe symptom. In the evaluation, a minimum of 8 points and a maximum of 40 points was considered. Patients who scored between 9 and 18 points reported mild nausea, and those who scored between 19 and 32 points reported moderate nausea. Those who scored between 33 and 40 points reported severe nausea, vomiting, retching, and pain, while patients with scores below 8 points did not report any of these symptoms. Three groups of patients were created based on this scoring: mild, moderate, and severe symptomatic categories.

### Statistical Analysis

The Shapiro-Wilk test was used to determine whether numerical variables conformed to a normal distribution. Three groups' normally distributed variables were compared using ANOVA and LSD tests, whereas three groups' non-normally distributed variables were compared using Kruskal-Wallis and Dunn tests. The cut-off point for vitamin D between the severe and mild hyperemesis groups was established using receiver operating characteristic (ROC) analysis. The analyses were conducted using MedCalc version 19.7.1 and SPSS version 22.0. A p-value of less than 0.05 was deemed significant.

## Results

Of the 80 patients in the research, 14 (17.5%) were classified as mildly symptomatic, 38 (47.5%) as moderately symptomatic, and 28 (35%) as severely symptomatic, based on the Rhodes Index score. Age, pregnancy, birth, abortion, number of live children, gestational weeks, and BMI did not significantly differ across the groups (Table 1).

Ketone positive rates in the mild, moderate, and severe symptomatic groups varied significantly ($p<0.05$). Thirteen (61.9%) and eight (38.1%) of the 21 patients who had the highest ketone positivity (+4) in the whole urine study were classified as very symptomatic and moderately symptomatic, respectively.

The groups' variations in vitamin D levels were statistically significant. The mild symptomatic group had vitamin D levels of 32.12±4.02 ng/mL; the moderate symptomatic group had 19.98±6.37 ng/mL; and the severe symptomatic group had 8.11±3.06 ng/mL ($p<0.001$).

A very strong negative correlation was detected between the Rhodes index score and vitamin D level ($r=-0.844$, $p=0.001$). In the ROC analysis, it was observed that the frequency and severity of nausea and vomiting increased when the vitamin D level of the serum was <11.54 ng/mL (Figure 1).

There were 23 (28.75%) patients with vitamin D deficiency (<10 ng/mL) and 22 (27.5%) patients with insufficiency (10-20 ng/mL). Of the patients with vitamin D levels at the deficiency level, 2 were in the moderate symptomatic group and 21 were

**Table 1.** Demographic characteristics of the HG patients

| | Mild (n=14) | Moderate (n=38) | Severe (n=28) | p |
|---|---|---|---|---|
| Age | 27.5±6.28 | 26.74±5.22 | 28.11±6.27 | 0.635 |
| Gravida | 3.21±1.72 | 2.76±1.6 | 3.25±1.88 | 0.462 |
| Number of pregnancies | 1.57±1.09 | 1.26±1.11 | 1.5±1.29 | 0.575 |
| Number of abortions | 0.93±1.21 | 0.61±0.82 | 0.86±0.93 | 0.433 |
| Number of living children | 1.5±1.02 | 1.13±1.02 | 1.39±1.17 | 0.464 |
| BMI | 25.96±5.03 | 23.5±3.04 | 24.24±3.38 | 0.265 |
| Gestational week | 9.57±1.34 | 9.84±1.94 | 10.07±2.04 | 0.737 |

*: Significant at $p<0.05$ level, BMI: Body mass index, HG: Hyperemesis gravidarum

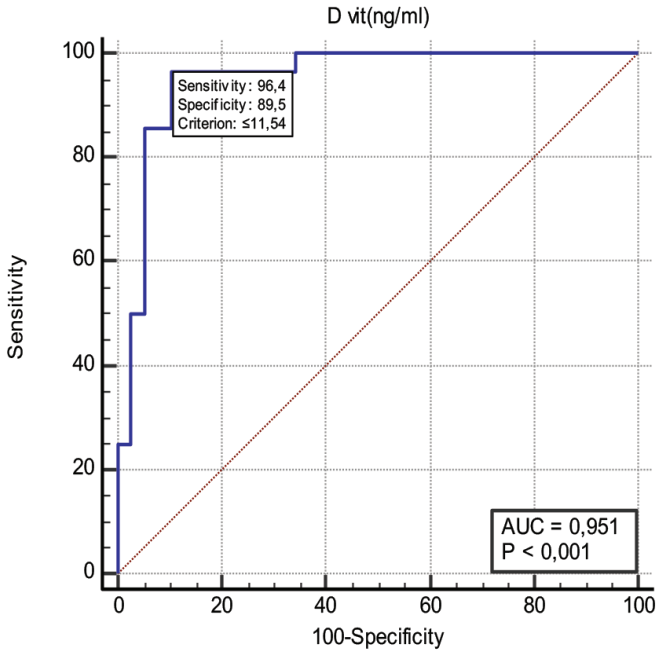


**Figure 1.** ROC analysis between the Rhodes index mean score and vitamin D level

*ROC: Receiver operating characteristic*

in the severe symptomatic group, while of the patients with another deficiency level, 15 were in the moderate symptomatic group and 7 were in the severe symptomatic group.

## Discussion

Due to its anti-inflammatory and immune-modulating properties, vitamin D plays a significant role in a number of reproductive disorders. Many immune cells possess vitamin D receptors. By inhibiting T-helper cell cytokine release, vitamin D promotes embryonic immune adaptation[17]. The inflammatory response is suppressed, and the placenta is prevented from secreting pro-inflammatory cytokines. Vitamin D insufficiency may be linked to a number of adverse outcomes for both women and babies, including hypertension, intrauterine growth restriction, spontaneous abortion, and preterm delivery, according to recent studies[18,19].

Inflammation and immunological dysregulation have been proposed as key factors in the etiopathogenesis of HG[20,21]. Vitamin D is an essential component of the reproductive system, an immune modulator, and anti-inflammatory agent. Thus, in this prospective analysis, we evaluated the hypothesis that pregnant women with HG should have reduced vitamin D levels. To the best of our knowledge, this is the first study in the body of literature to use an objective scale to rate vitamin D levels and HG symptoms. Given that vitamin D is known to have a variety of roles in the reproductive system, this study suggests it may also have an impact on HG.

According to HG symptoms, we aimed to determine whether vitamin D levels varied across groups with mild, moderate, and severe symptoms. In contrast to previous research in the literature, we attempted to establish an objective symptom degree in our study by using the Rhodes index to score the intensity of symptoms and grading the responses provided by HG pregnant women. As a result, we separated the patients into three groups: those with mild symptoms, those with moderate symptoms, and those with severe symptoms. We found that the patients' vitamin D levels dramatically dropped as their symptom levels rose. In summary, we discovered a negative correlation between the intensity of HG symptoms and serum vitamin D levels. In light of these findings, it is recommended that future research explore the potential benefits of investigating serum vitamin D levels in pregnant women experiencing severe symptoms, including nausea and vomiting. This investigation should also include the administration of appropriate medications to women with serum vitamin D deficiency or insufficiency prior to becoming pregnant. The objective of this approach is to determine whether reducing the severity and frequency of HG symptoms in pregnant women can be effectively achieved.

While we did not assess serum vitamin D levels between pregnant women with HG and normal pregnant women without nausea and vomiting, in our study, our primary objective was to categorize patients based on their symptoms of HG and to ascertain vitamin D levels in each group. This approach would facilitate the interpretation of the severity of symptoms in relation to vitamin D levels. A critical next step is to determine whether vitamin D affects the etiopathogenesis of HG, or whether inflammation and the underlying cause of the disease lead to decreased vitamin D levels. Consequently, further studies investigating the relationship between vitamin D and HG in larger numbers of patients are needed. In designing these studies, it would be advantageous to categorize HG patients based on the severity of their symptoms using objective criteria and to establish a group that compares the vitamin D levels of these categories with the vitamin D levels of normal pregnant women. The exclusion of pregnant women who did not receive a HG diagnosis from our study could be regarded as a study limitation.

HG-related morbidities make it a significant medical concern. Fetomaternal morbidity rises, and metabolic and nutritional abnormalities linked to the severity of HG are prone to develop[22]. With over 59,000 hospital admissions annually, HG is the leading cause of hospitalization during the first part of pregnancy[23,24]. Over the course of pregnancy, HG causes consultations, ED visits, and hospitalizations[25-27]. According to conservative estimates, NVP cost the US economy more than $7 billion in 2012, with direct expenses of more than $1 billion[28]. Indirect costs include lost work and caregiver time. This total of more than $700 million is likely to be an underestimate, as not all applicable costs can be included.

In the present study, we observed a significant difference in vitamin D levels among the mild, moderate, and severe symptomatic groups. Furthermore, a direct correlation was identified between the decrease in vitamin D levels and the escalation in symptom severity among patients. The area under the curve analysis revealed that patients with serum vitamin D levels below 11.54 ng/ml exhibited a substantial increase in symptoms with 96% sensitivity and 89% specificity. In a study by Sahin et al.[29], serum 25OHD3 levels were found to be significantly lower in the severe HG group compared to the control group. The observed outcomes were attributed to inadequate calcium and vitamin D intake, a condition precipitated by symptoms such as nausea and vomiting. The existing literature documents deficiencies in thiamine, riboflavin, vitamin B6, vitamin A, retinal binding protein, and vitamin K in over 60% of these patients[28].

Vitamin D deficiency is prevalent among adolescents and particularly notable among African Americans, with approximately 40% of African Americans and 4% of non-Hispanic Caucasian women having low plasma concentrations of Vitamin D. The vitamin D status of pregnant women is also a concern, even in industrialized countries. Research has shown that moderately reduced 25(OH)D levels in late winter can lead to poor skeletal development and tooth mineralization in the fetus and newborn[12]. The use of vitamin D supplements in excess of the prescribed levels to avoid deficiency during pregnancy is not currently supported by any data[13]. Higher dosages of vitamin D supplementation, however, may be beneficial for pregnant women who have severe nausea and vomiting, according to our study and the study conducted by Sahin et al.[29].

Pregnant women are often advised to take a multivitamin that contains minerals before giving birth. If taken at least one month prior to pregnancy, this may lessen the frequency and severity of NVP[30]. Malnutrition and excessive electrolyte losses put women with HG at risk for nutritional deficiencies[31]. During pregnancy, vitamin D insufficiency, hypovitaminosis D, and inadequate sun exposure are prevalent[32,33]. Several studies have found that the Greek population has a significant frequency of vitamin D insufficiency and hypovitaminosis D, despite Greece being one of the sunniest nations in Europe[34]. A deficit prevalence of 23-90% was found in a comprehensive assessment of women in Mediterranean nations, including pregnant women[35]. This might have detrimental effects on the health of both the mother and the newborn. According to a study conducted in our nation, 8-61% of people were vitamin D deficient[36]. Similarly, in healthy pediatric age groups, research from throughout the world revealed that 7-68% had vitamin D deficiency and 19-61% had vitamin D insufficiency[37].

The ideal values of 25(OH)D are a matter of debate[38,39]. A 25(OH)D level below 20 ng/mL is regarded as a sign of vitamin D insufficiency. However, values between 20-30 ng/mL are deemed inadequate by the Endocrine Society and other expert organizations. Reaching concentrations beyond 40 ng/mL is advised by certain publications. Due to its possible role in fertility, there is now more evidence supporting the necessity of vitamin D both before and throughout pregnancy. Worldwide, vitamin D insufficiency is quite prevalent in pregnant women in every stage of pregnancy, according to several research studies[40-42].

The concentration of 25(OH)D in pregnant women has been found to be influenced by skin pigmentation, UV rays, and avoiding sun exposure due to cultural or religious beliefs. There is a significant risk of nutritional deficit in pregnant women experiencing winter pregnancy and obesity (BMI >30 kg/m$^2$)[43]. Pregnancy-related hypovitaminosis D is quite prevalent around the world[44]. Low vitamin D levels in pregnant Asian women were quite common, according to a study by Palacios et al.[45]. In India, 96% of the population; in China, 69%; in Kuwait, 70% to 83% of the population; in Pakistan, 72%; in Iran, 67%; and in Türkiye, 90% have the condition. The prevalence of vitamin D insufficiency is also very high. Research has demonstrated that within the Turkish population, 50% is affected, whereas in Pakistan, the figure stands at 45%. In Kuwait, the prevalence ranges from 38% to 41%, and in India, it has been documented as high as 60%. A recent review of 13 studies from seven countries found that the prevalence of vitamin D deficiency and insufficiency ranged from 39.4% to 76.5%. Vitamin D deficiency has been identified as a pervasive public health concern, impacting a substantial proportion of the global population. Notably, pregnant women are particularly vulnerable to this deficiency[46-49]. To benefit from the positive effects of vitamin D, it is recommended to maintain serum concentrations between 30 and 50 ng/mL[50-53]. In Türkiye, all women receive free vitamin D supplementation (1,200 IU/day) from early pregnancy until six months after delivery[54]. The global prevalence of vitamin D deficiency among pregnant women ranges from 20% to 40%, with figures ranging from 18.2% to 45.9% observed in Türkiye[55,56].

### Study Limitations

The limited number of patients in each group and the exclusion of vitamin D levels in pregnant women without HG are two of the study's limitations. It is crucial, therefore, to establish a reference for research including pregnant women who do not have HG.

## Conclusion

This study found significant differences in vitamin D concentrations among groups experiencing mild, moderate, and severe HG symptoms. According to the results, more severe HG symptoms are linked to lower vitamin D levels. Our study suggested that checking vitamin D levels before pregnancy and providing a routine replacement for patients with deficiency and insufficiency levels might help prevent severe HG symptoms. This can reduce hospitalizations and economic expenditures

related to this issue. HG patients cannot be treated on an outpatient basis and require long-term hospitalization.

### Ethics

**Ethics Committee Approval:** The Faculty of Medicine Gaziantep University Clinical Research Ethics Committee granted approval for this study, which was carried out in compliance with the Helsinki Declaration's criteria (approval number: 241/2020; date: 16.07.2020).

**Informed Consent:** Consent form was filled out by all participants.

### Footnotes

### Authorship Contributions

Surgical and Medical Practices: N.B.T., H.Ç.Ö., Concept: N.B.T., Design: N.B.T., D.B., Data Collection or Processing: N.B.T., R.G., H.T., Analysis or Interpretation: N.B.T., H.Ç.Ö., T.G.K., Literature Search: N.B.T., R.G., Writing: N.B.T., D.B.

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

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# Artificial intelligence in prenatal diagnosis: Down syndrome risk assessment with the power of gradient boosting-based machine learning algorithms

## *Prenatal tanıda yapay zeka: Gradient boosting tabanli makine öğrenmesi algortimalarinin gücü ile Down sendromu risk değerlendirmesi*

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### Abstract

**Objective:** One of the most common chromosomal abnormalities seen during pregnancy is Down syndrome (Trisomy 21). To determine the risk of Down syndrome, first-trimester combined screening tests are essential. Using data from the first-trimester screening test, this study compares machine learning and deep learning models to forecast the risk of Down syndrome.

**Materials and Methods:** Within the scope of the study, biochemical and biophysical data of 959 pregnant women who underwent first-trimester screening tests at Çukurova University Obstetrics and Gynecology Clinic between 2020-2024 were analyzed. After cleaning missing and erroneous data, various preprocessing and normalization techniques were applied to the final dataset consisting of 853 observations. Down syndrome risk prediction was performed using different machine learning models, and model performances were compared based on accuracy rates and other evaluation metrics.

**Results:** Experimental results show that the CatBoost model provides the highest success rate, with an accuracy rate of 95.31%. In addition, the XGBoost and LightGBM models exhibited high performance, with accuracy rates of 95.19% and 94.84%, respectively. The study also examines the effects of the class imbalance problem on model performance in detail and evaluates various strategies to reduce this imbalance.

**Conclusion:** The findings show that gradient boosting-based machine learning models have significant potential in Down syndrome risk prediction. This approach is expected to contribute to the reduction of unnecessary invasive tests and improve clinical decision-making processes by increasing the accuracy rate in prenatal screening processes. Future studies should aim to increase the generalization capacity of the model on larger data sets and to provide integration with different machine learning algorithms.

**Keywords:** Down syndrome, first-trimester screening test, gradient boosting, machine learning, artificial intelligence, classification algorithms

**PRECIS:** The aim of the study is to increase the accuracy rate in prenatal screening processes, and it is expected to contribute to reducing unnecessary invasive tests and improving clinical decision-making processes.

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**Öz**

**Amaç:** Down sendromu (Trizomi 21), prenatal dönemde en sık rastlanan kromozomal anomalilerden biridir. Gebeliğin birinci trimesterinde uygulanan kombine tarama testleri, Down sendromu riskinin belirlenmesi için önemli bir araç olarak kullanılmaktadır. Bu çalışma, birinci trimester tarama testi verileri kullanılarak Down sendromu riskini tahmin etmek amacıyla farklı makine öğrenmesi ve derin öğrenme modellerini karşılaştırmalı olarak değerlendirmeyi amaçlamaktadır.

**Gereç ve Yöntemler:** Çalışma kapsamında, 2020-2024 yılları arasında Çukurova Üniversitesi Kadın Doğum Kliniği'nde birinci trimester tarama testine tabi tutulan 959 gebeye ait biyokimyasal ve biyofiziksel verileri analiz edilmiştir. Eksik ve hatalı veriler temizlendikten sonra, 853 gözlemden oluşan nihai veri seti üzerinde çeşitli ön işleme ve normalizasyon teknikleri uygulanmıştır. Farklı makine öğrenmesi modelleri kullanılarak Down sendromu risk tahmini gerçekleştirilmiş, model performansları doğruluk oranları ve diğer değerlendirme metrikleri üzerinden karşılaştırılmıştır.

**Bulgular:** Deneysel sonuçlar, CatBoost modelinin %95,31 doğruluk oranı ile en yüksek başarıyı sağladığını göstermiştir. Bunun yanı sıra, XGBoost ve LightGBM modelleri sırasıyla %95,19 ve %94,84 doğruluk oranları ile yüksek performans sergilemiştir. Çalışmada ayrıca sınıf dengesizliği probleminin model performansı üzerindeki etkileri detaylı olarak incelenmiş ve bu dengesizliği azaltmaya yönelik çeşitli stratejiler değerlendirilmiştir.

**Sonuç:** Elde edilen bulgular, gradient boosting tabanlı makine öğrenmesi modellerinin Down sendromu risk tahmininde önemli bir potansiyele sahip olduğunu göstermektedir. Bu yaklaşımın, prenatal tarama süreçlerindeki doğruluk oranını artırarak, gereksiz invaziv testlerin azaltılmasına ve klinik karar alma süreçlerinin iyileştirilmesine katkı sağlaması beklenmektedir. Gelecekteki çalışmalar, daha geniş veri setleri üzerinde modelin genelleştirme kapasitesini artırmayı ve farklı makine öğrenmesi algoritmalarıyla entegrasyon sağlamayı hedeflemelidir.

**Anahtar Kelimeler:** Down sendromu, ilk trimester tarama testi, gradyan güçlendirme, makine öğrenmesi, yapay zeka, sınıflandırma algoritmaları

## Introduction

Down syndrome (DS) is one of the most common chromosomal abnormalities in humans. It affects individuals regardless of race, age, or socioeconomic status. The condition occurs due to a genetic anomaly in which an extra chromosome is present in the 21st pair, resulting in a total of 47 chromosomes. The incidence rate is estimated to be approximately 1 in every 600 to 800 live births[1,2]. DS is associated with various physical, cognitive, and developmental challenges, along with a range of health complications. Studies have shown that increasing maternal age significantly elevates the risk of DS. Nevertheless, early diagnosis and appropriate management are achievable through prenatal screening tests and genetic counseling.

First-trimester screening (FTS) is a fundamental method for the early detection of DS. This approach integrates maternal serum biomarkers, including free beta-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein A (PAPP-A), with ultrasound-based parameters such as nuchal translucency (NT), crown-rump length (CRL), and the absence or presence of the nasal bone. NT measurements are typically performed between the 11th and 14th weeks of gestation. In cases of DS, β-hCG levels are often elevated, whereas PAPP-A levels tend to be reduced. In contrast, Trisomy 18 and Trisomy 13 are generally associated with lower levels of both markers[3,4].

Accurate interpretation of screening results requires a clear understanding of the multiple of the median (MoM) method. This approach standardizes test values by dividing each measurement by the median value corresponding to the specific gestational week[5]. Indicators of high risk for DS include a NT measurement greater than 2.5 millimeters, absence of the nasal bone, a PAPP-A level below 0.4 MoM, and a β-hCG level above 2.5 MoM. When NT is 3 millimeters or greater or exceeds the 99th percentile, further fetal evaluation and genetic counseling are strongly recommended. In such cases, additional risk assessment using cell-free fetal DNA (cfDNA) analysis and confirmatory diagnostic testing should also be considered[6].

Combined screening tests performed between the 11th and 14th weeks of pregnancy typically yield a false-positive rate of approximately 5 percent and an overall accuracy rate approaching 90 percent. Based on these results, risk levels are categorized as high (equal to or greater than 1 in 250), moderate (between 1 in 250 and 1 in 1000), or low (equal to or less than 1 in 1000)[7,8]. If the screening results are abnormal, amniocentesis is usually recommended. This invasive procedure, typically conducted between the 15th and 20th weeks of gestation, involves extracting fetal cells from the amniotic fluid for genetic analysis[9]. Although it is considered a reliable diagnostic method, amniocentesis carries a small risk of fetal loss. These risks, while infrequent, emphasize the importance of developing more accurate and non-invasive alternatives[10].

In predictive classification, the combination of NT measurements with serum biomarkers enhances the accuracy of DS risk assessment. Artificial intelligence (AI) models are capable of identifying complex patterns within such data, allowing for more precise classification of risk levels[11]. AI, particularly through machine learning (ML) and deep learning (DL) approaches, facilitates the analysis of large and complex datasets across various disciplines[12]. In the field of healthcare, these technologies have led to faster diagnoses and more efficient treatment planning[13].

Conventional DS screening methods may be subject to errors due to limitations in clinical expertise or access to advanced technology. In some cases, families may also decline NT measurement because of cultural or personal beliefs. This study integrates both biophysical markers (NT) and biochemical indicators (hCG and PAPP-A) to assess DS risk. The primary objective is to develop a model that reduces the impact of geographic variability and increases the robustness of predictions despite potential test inaccuracies. Data were collected from

959 singleton pregnancies at Çukurova University between 2020 and 2024. After preprocessing, the dataset was used to train AI-based classification models. The outcomes aim to enhance diagnostic accuracy and assist clinicians in prenatal risk evaluation and decision-making.

## Materials and Methods

This study effectively estimates the risk of DS, contributing to the health and general well-being of both the mother and the unborn child. The following sections comprehensively explain the applied methodological approaches and present the findings, demonstrating the accuracy and clinical significance of the results.

### Dataset and Preprocessing

This study analyzed data obtained from the combined double screening tests of 959 women with singleton pregnancies in the first trimester at the Obstetrics and Gynecology Unit of Çukurova University between 2020 and 2024. The study protocol and data collection process were reviewed and approved by the Çukurova University Faculty of Medicine Research Ethics Committee in accordance with ethical standards (approval number: 144, date: 10.05.2024). Patient records were retrieved from the hospital's gynecology and obstetrics clinic as well as the biochemistry laboratories. To ensure confidentiality and compliance with ethical regulations, all patient data were anonymized, and no personally identifiable information was used at any stage. Details of the dataset and the variables included in the analysis are presented in Table 1.

Prior to the development of the AI model for risk estimation, several preprocessing steps were applied to the dataset to address missing data and improve data quality. Erroneous entries were corrected, and records with duplicate or missing values were excluded. As a result, the initial dataset of 959 records was reduced to 853 valid entries. To improve model accuracy, the distribution of the target variable (DS risk class) was examined. The final dataset included 195 samples (22.9%) classified as medium risk, 474 samples (55.6%) as low risk, and 184 samples (21.6%) as high risk. The categorical distribution of the target variable is illustrated in Figure 1.

### Normalization[14]

The normalization process was used to improve the model's accuracy and stability because the data set's of the independent variables in the study's data set varying value ranges could cause a scale difference between the variables. Normalization contributes to the stable and efficient operation of ML algorithms by ensuring that the variables are represented on the same scale. Different transformation techniques were evaluated for the target and independent variables in the data preprocessing stage, and appropriate methods were determined.

In particular, since the target variable is categorical, the label encoding method was preferred for the appropriate transformation of the classes. This method allows ML algorithms to process categorical variables more effectively by converting them to numerical values. In the scaling process of the independent variables, three different normalization techniques were tested:

**Minimum-Maximum Scaler:** This method scales variables to a specific range (usually between 0 and 1) to ensure all features remain within the same limits. Min-max scaling is especially effective when the data is concentrated in a particular range and is preferred when distribution distortion needs to be prevented.

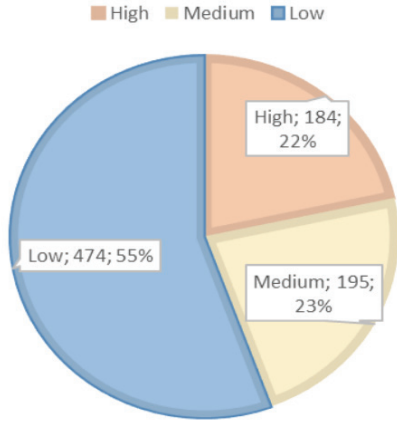


**Figure 1.** Distribution of the Down syndrome attribute in basic dataset

**Table 1.** The attributes explanation of the collected dataset

| Attribute | Description | Data type |
|---|---|---|
| hCG-MoM | Human chorionic gonadotropin (hCG), method of medians (MoM). hCG is a hormone secreted by the placenta during pregnancy and is found at high levels in the early stages of pregnancy. The MoM value is obtained by dividing the measured value by the average value for the corresponding gestational week. | Numeric |
| PAPP-A MoM | Pregnancy-associated plasma protein A (PAPP-A) is a protein produced by the placenta during pregnancy, known as PAPP-A. | Numeric |
| NT | Nuchal translucency (NT) is the measurement of the thickness of the fetal neck obtained via ultrasound. | Numeric |
| NT MoM | It is calculated by dividing the NT value by the average value for the corresponding gestational week. | Numeric |
| DS | Down syndrome risk classification is as follows: a ratio below 1:250 is considered high risk (high), between 1:250 and 1:1000 is classified as medium risk (medium), and above 1:1000 is categorized as low risk (low). | Categorical |

**Standard Scaler:** This method standardizes the mean of the variables to 0, and the standard deviation to 1, resulting in a standard normal distribution. It is an effective technique, especially for variables with a normal distribution, and is widely used for many ML algorithms.

**Robust Scaler:** This approach, which applies data scaling based on the median and interquartile range, was designed to minimize sensitivity to outliers. Transforming the data using central tendency measures reduces the negative impact of extreme values on the model. Comparisons conducted within the scope of the study revealed that the Robust Scaler method yielded the most successful results, particularly in cases where outliers were present in the dataset.

As a result of this process, the scaling of the data set was completed, and the model's performance was intended to improve. The normalization process allows the model to learn faster and more stably while improving its prediction performance.

### AI Classification Models

In this study, a comprehensive selection of AI-based classification models was utilized to evaluate their predictive performance in assessing DS risk. The models were chosen based on their demonstrated effectiveness in addressing class imbalance, capturing complex non-linear relationships among variables, and performing well in clinical risk classification contexts.

The classifiers were selected with careful consideration of the dataset's characteristics, including its numerical structure, class imbalance, and risk-based output labels. Tree-based ensemble methods such as CatBoost, XGBoost, and LightGBM were included due to their ability to handle structured clinical data effectively. These models are known for their robustness against outliers, high predictive accuracy, and efficient processing in large datasets. Their successful application in previous prenatal and healthcare-related classification tasks further supports their appropriateness for this study.

An overview of the models and their technical characteristics is provided in Table 2.

### Proposed Approach

This study proposes a novel diagnostic approach for early DS risk assessment by integrating biochemical markers (hCG and PAPP-A) and a biophysical parameter (NT) obtained from the combined FTS. These markers were selected based on their well-established roles in prenatal screening. While hCG and PAPP-A provide insights into biochemical deviations associated with chromosomal abnormalities, NT offers a structural sonographic dimension. Combining these complementary features enhances the reliability of early risk estimation.

The novelty of the proposed method lies in the application of advanced AI-based classification models, which go beyond the static threshold-based decisions of traditional screening tools. Unlike conventional methods, the AI-supported approach can capture complex, non-linear interactions between features, enabling more precise and individualized risk stratification. This is particularly important in cases where conventional cut-off values may misclassify borderline or atypical presentations.

In addition, the proposed model addresses specific limitations such as operator dependency in NT measurements and potential false reassurance in low-risk cases. By leveraging the learning capabilities of ML algorithms, the model contributes to improving diagnostic robustness and reducing unnecessary invasive procedures.

The general architecture of the proposed methodology is presented in Figure 2.

**Table 2.** Classification models used in the study and their explanations

| Model | Algorithm category | Framework/library | Purpose |
|---|---|---|---|
| Extreme gradient boosting[15] | Gradient boosting | XGBoost | Classification tasks with high performance. |
| Light gradient boosting machine[16] | Gradient boosting | LightGBM | Efficient gradient boosting for large datasets. |
| Categorical boosting (CatBoost)[17] | Gradient boosting | CatBoost | Handles categorical features automatically. |
| K-nearest neighbors (KNN)[18] | Instance-based | scikit-learn | Simple classifier based on distance metrics. |
| Naive bayes (NB)[19] | Probabilistic | scikit-learn | Based on Bayes' theorem, handles continuous data. |
| Logistic regression (LR)[20] | Linear model | scikit-learn | Binary and multiclass classification. |
| Support vector machine (SVM)[21] | Support vector machine | scikit-learn | Classification with margin maximization. |
| Decision tree (DT)[22] | Tree-based | scikit-learn | Simple, interpretable decision rules. |
| Random forest (RF)[23] | Ensemble (bagging) | scikit-learn | Combines multiple decision trees for accuracy. |
| Adaptive boosting (AdaBoost)[24] | Ensemble (boosting) | scikit-learn | Boosting technique to improve weak classifiers. |
| CNN[12] | Deep learning | TensorFlow/Keras | Pattern recognition in high-dimensional data. |
| LSTM[12] | Deep learning (recurrent neural networks) | TensorFlow/Keras | Sequence modeling for time series or text. |

## Results

The study used ten distinct ML and two DL classifiers to evaluate the suggested strategy's effectiveness. The k-fold cross-validation method (k=5) was selected to assess the ML classifiers. Accuracy, Precision, Recall, and F1-score, among the widely used evaluation criteria, were used as the performance scale of the ML classifiers[12,25].

As can be seen in Figure 3, the highest accuracy performance was obtained with Boosting-based methods. After the comparison, CatBoost obtained the best performance with an accuracy of 95.31%. XGBoost came in second with 95.19%, and LightGBM came in third with 94.84%. In this study, which primarily worked with numerical data, tree-based ML models such as CatBoost and XGBoost were preferred due to their robustness against outliers and strong generalization capacity. These models exhibit high performance in learning complex relationships, different features in the dataset, and can capture interactions between variables. In addition, imbalances in the class distribution in the dataset can be managed more effectively thanks to the flexible structure of tree-based models. Such models can make more balanced and adaptable predictions for each class through the use of decision trees to determine patterns in the dataset. In addition, the LightGBM model works with high speed, and low memory usage on large data sets, making it practical to prefer this model.

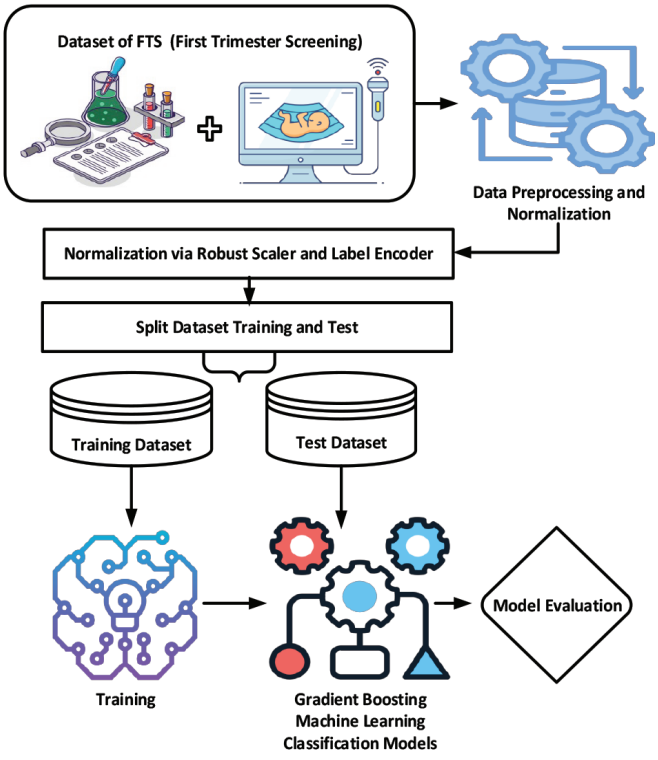


**Figure 2.** Flowchart of the proposed approach

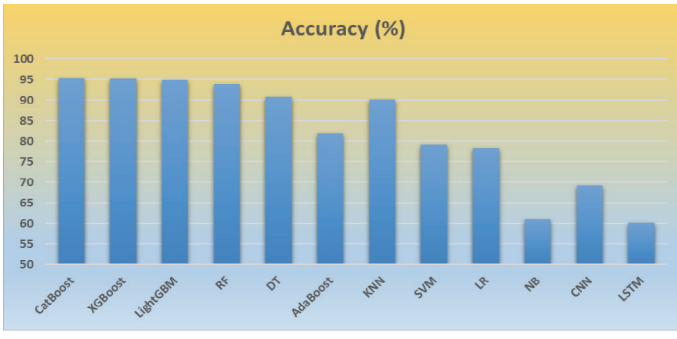


**Figure 3.** Accuracy performance comparison of classification models

Because of the unequal distribution of classes, it is important to look at the metrics for each class separately. It is essential to analyze the performance metrics specific to each class to understand the effectiveness of the ML models used in this study. Specifically, for imbalanced datasets, the model's capacity to discriminate between classes may vary, substantially impacts performance metrics. To evaluate the model's overall efficacy and performance for each class, accuracy, recall, precision, and F1 scores were carefully examined. The following graphs were created to enable more accurate comparison and visual depiction of the model's performance by class. By highlighting the model's advantages and disadvantages for various classes, these visualizations provide insights for improvement strategies. As seen in Figure 4, the overestimation of the low class and the underestimation of the other classes are due to the imbalance of the class distribution in the dataset and how the model adapts to this imbalance. This is critical in understanding the model's learning bias towards certain classes, especially when working with imbalanced datasets. An imbalanced class distribution can cause the model to give more weight to the majority class and fail to learn rare classes well enough. Therefore, when evaluating the model's success for each class, it is important to examine how the predictions are distributed on a class basis. Different balancing techniques should be applied in line with these results, and performance improvement strategies should be developed to increase the model's sensitivity to imbalanced datasets.

## Discussions

This study introduces a ML approach to predict the risk of DS using first-trimester combined screening test (FTS) data. The dataset analysis involves comparing various ML models, incorporating biochemical (hCG, PAPP-A) and biophysical (NT) parameters. AI has changed the world's agenda in recent years, and its use will become increasingly widespread in all areas in the coming years. There are few publications in the literature about the use of AI in obstetrics.

Neocleous et al.[26] developed an AI model that utilizes various features to assess the risk of aneuploidy and other chromosomal abnormalities. This model incorporates several variables, such

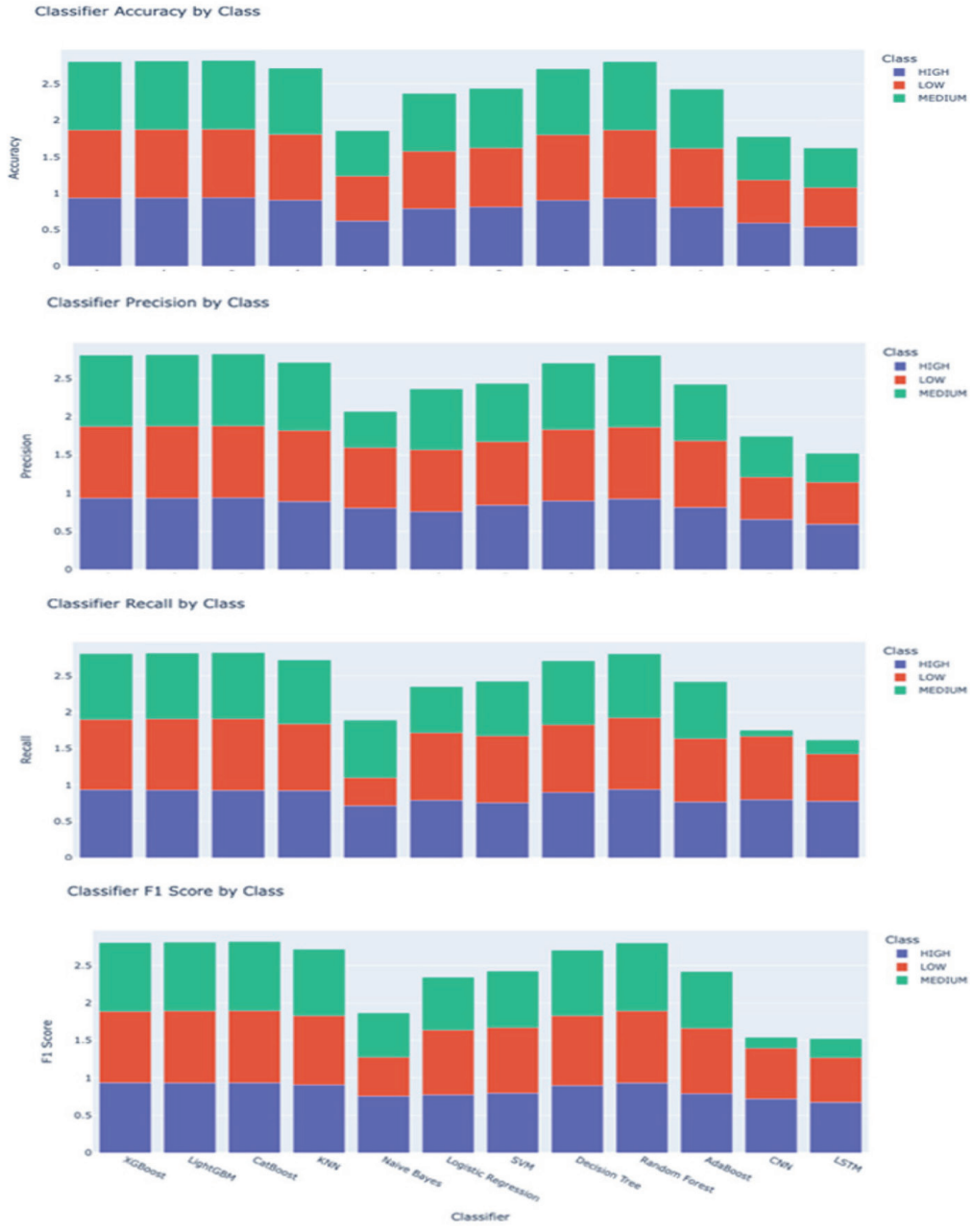


**Figure 4.** Performances of the classification models based on the proposed approach according to Down syndrome classes

as maternal age, the presence of a nasal bone, biochemical markers, (β-HCG, PAPP-A MoM), ultrasound measurements during pregnancy (CRL), NT, and a history of DS in prior pregnancies. These parameters were examined using ML algorithms to determine the probability of fetal abnormalities. The study emphasizes how important, ML is for identifying and evaluating the risks associated with chromosomal abnormalities. Koivu et al.[27] used the support vector machine (SVM) model to improve the precision of fetal DS screening. The SVM algorithm works exceptionally well with multidimensional data, making it ideal for intricate analyses such as identifying fetal abnormalities. Meanwhile, Subasi[20] conducted fetal aneuploidy screening using non-invasive prenatal testing (NIPT) and fetal DNA (cffDNA) found in maternal blood. Their study significantly contributed to advancements in prenatal screening techniques by improving the accuracy of genetic tests and offering noninvasive alternatives to traditional diagnostic methods.

Durmuşoğlu et al.[11] proposed a ML model based on triple test indicators obtained from Gaziantep University, Turkey. This model aims to obtain more reliable results by avoiding the adverse effects of the triple test. The authors used nine ML models in the study and applied the SMOTE technique to generate synthetic data due to insufficient datasets. This

technique eliminated dataset imbalance and increased the model's accuracy.

Uzun and Kaya[28] developed an ML model, including Bayesian and naive Bayesian algorithms, using biochemical and biophysical FTS measurements to detect Trisomy 21. Since the dataset used in this study contained sample deficiencies and imbalances, the authors resorted to optimization techniques. This approach increased the model's performance and provided accurate results.

Catic et al.[29] proposed a neural network-based model for detecting DS and other genetic disorders (Edwards, Turner, Klinefelter Syndrome, Patau) using maternal serum screening data in the first trimester. They used a dataset of 2500 samples in their study, and the experimental results showed that recurrent neural networks provide higher accuracy than other methods.

Wøjdemann et al.[30] used combined test data (NT and double test) and double test data (β-hCG, PAPP-A) to examine the detection of DS and other chromosomal abnormalities in a Danish population. The study aimed to enhance screening accuracy by evaluating the effectiveness of different test combinations. The findings highlight the significance of both dual and combined tests as essential tools for the early detection of chromosomal abnormalities.

These studies are important illustrations of the potential applications of technologies such as ML, AI, and DL in the healthcare industry, specifically in genetic disorder detection and prenatal screening. The high accuracy rates of the developed models can help reduce false positives and increase the dependability of screening results.

The study results show that machine learning-based models can be used effectively in prenatal screening processes and that higher accuracy rates can be achieved compared to traditional methods. Such AI-supported approaches can contribute to reducing unnecessary invasive tests and improving clinical decision-making processes by increasing early diagnosis accuracy. Future studies should aim to increase the generalization capacity of the model on larger data sets and to provide integration with different ML algorithms.

### Study Limitations

This study has several limitations that should be acknowledged. First, although the dataset initially included 959 records, 106 cases (approximately 11%) were excluded due to missing or duplicate data. Even though data cleaning was carefully performed, this reduction may have caused a minor loss in statistical power. Internal comparisons between complete-case models and those with imputed data showed a slight average difference of 1.3% in accuracy. This suggests that missing data might have had a modest impact on model performance.

Second, due to the earthquake on February 6, 2023, our hospital was temporarily evacuated, and routine data archiving was disrupted. While test results before this date were systematically recorded in the WePoint system along with ultrasound images, the records after the earthquake lacked these images. As a result, NT measurements in 318 cases could not be verified. Since NT is operator-dependent, this may have introduced measurement inconsistencies that could affect the model's accuracy.

Third, although the sample size was relatively large (n=853), all data were collected from a single tertiary hospital in southeastern Türkiye. Therefore, the findings may not fully represent populations from different geographic or clinical settings. Model performance may vary elsewhere, and recalibration could be needed before applying it in other regions.

Fourth, the study did not include diagnostic confirmation through invasive tests such as amniocentesis, nor did it include non-invasive tests like NIPT. Therefore, sensitivity, specificity, positive predictive value, and negative predictive value could not be calculated. In addition, follow-up data on post-screening clinical decisions were not available, limiting our ability to evaluate the real-world impact of the model.

Finally, this model was developed using only first-trimester biochemical and biophysical markers. Other clinical factors such as maternal health conditions, lifestyle habits, or family history were not included. Future research should include a wider range of clinical data and involve prospective, multicenter studies to improve generalizability and clinical usefulness.

## Conclusion

Experimental findings indicate that tree-based ML models demonstrated superior performance, particularly in the presence of class imbalance within the dataset. Among the evaluated models, CatBoost achieved the highest accuracy rate at 95.31 percent, followed closely by XGBoost at 95.19 percent and LightGBM at 94.84 percent. These models were especially effective in capturing complex relationships among variables and showed strong generalization capabilities. In contrast, more traditional classification algorithms yielded comparatively lower accuracy scores, suggesting their limited capacity to handle the non-linear patterns and imbalance inherent in the data.

Further class-based performance analyses revealed that the dataset exhibited a skewed distribution, with a disproportionately high number of samples in the low-risk category. As a result, the models tended to overpredict the low-risk class while underrepresenting medium- and high-risk groups. This observation highlights a potential bias introduced by the class imbalance, which may compromise the model's sensitivity in detecting higher-risk cases.

To address this limitation, the integration of advanced data balancing techniques, such as oversampling, undersampling, or synthetic data generation, (e.g., SMOTE) is recommended for future research. Incorporating these methods may help enhance the model's performance across all risk groups, thereby improving both the fairness and diagnostic value of AI-supported prenatal risk assessments.

### Ethics

**Ethics Committee Approval:** The study protocol and data collection process were reviewed and approved by the Çukurova

University Faculty of Medicine Research Ethics Committee in accordance with ethical standards (approval number: 144, date: 10.05.2024).

**Informed Consent:** Documents of informed consent were taken from all patients.

### Footnotes

### Authorship Contributions

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# Evaluation of lymph node metastasis in cervical cancer: A retrospective comparison of preoperative MRI and PET/CT with postoperative histopathology results

## *Serviks kanserinde lenf nodu metastazının değerlendirilmesi: Preoperatif MRG ve PET/BT ile postoperatif histopatoloji sonuçlarının retrospektif olarak karşılaştırılması*

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### Abstract

**Objective:** The aim of this study is to assess the diagnostic performance of positron emission tomography/computed tomography (PET/CT) and magnetic resonance imaging (MRI) in detecting pelvic and paraaortic lymph node involvement in cervical cancer patients by correlating imaging results with surgical pathology findings.

**Materials and Methods:** A retrospective analysis was conducted on cervical cancer patients treated at İstanbul Medeniyet University Prof. Dr. Süleyman Yalçın City Hospital from 2016 to 2022. Patients who underwent preoperative PET/CT or MRI imaging and subsequent lymph node dissection were included. Sensitivity, specificity, positive predictive value, and negative predictive value were calculated for each imaging modality.

**Results:** Of the 75 cases reviewed, 52 met the inclusion criteria. PET/CT had higher specificity (94.1%) than MRI (82.4%), while MRI demonstrated greater sensitivity (55.6% vs. 50%). False-negative rates were 15.3% for MRI and 17.3% for PET/CT. Receiver operating characteristic analysis indicated an area under the curve of 0.78 for PET/CT and 0.69 for MRI. No statistically significant differences in sensitivity or specificity were observed, with both modalities showing complementary strengths.

**Conclusion:** MRI and PET/CT each contribute significantly to preoperative cervical cancer evaluation, with MRI favored for local assessment and PET/CT for nodal detection. Combining both modalities enhances diagnostic accuracy. Further prospective research is required to confirm and strengthen these results. and improve imaging strategies for clinical practice.

**Keywords:** Lymph node, MRI, PET/CT

### Öz

**Amaç:** Bu çalışma, serviks kanseri hastalarında pelvik ve paraaortik lenf nodu metastazlarının saptanmasında pozitron emisyon tomografisi/bilgisayarlı tomografi (PET/BT) ve manyetik rezonans görüntülemenin (MRG) tanısal doğruluğunu araştırmakta ve görüntüleme bulgularını postoperatif histopatoloji ile karşılaştırmaktadır.

**Gereç ve Yöntemler:** 2016-2022 yılları arasında İstanbul Medeniyet Üniversitesi Prof. Dr. Süleyman Yalçın Şehir Hastanesi'nde tedavi edilen serviks kanseri hastalarının retrospektif analizi yapılmıştır. Preoperatif PET/BT veya MRG görüntülemesi ve ardından lenf nodu diseksiyonu yapılan hastalar çalışmaya dahil edilmiştir. Her bir görüntüleme yöntemi için duyarlılık, özgüllük, pozitif prediktif değer ve negatif prediktif değer hesaplanmıştır.

**PRECIS:** Positron emission tomography/computed tomography and magnetic resonance imaging complement each other in detecting cervical cancer lymph node metastasis, with false negativity rates of 15.3% and 17.3%, respectively. Their combination improves accuracy and warrants further research.

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**Bulgular:** İncelenen 75 olgudan 52'si dahil edilme kriterlerini karşıladı. PET/BT, MRG'ye kıyasla daha yüksek özgüllüğe (%94,1) sahipken, MRG daha yüksek duyarlılık (%55,6'ya karşı %50) gösterdi. Yanlış negatiflik oranları MRG için %15,3, PET/BT için %17,3 olarak bulundu. Alıcı işletim karakteristiği analizi, PET/BT için eğri altındaki alanın 0,78, MRG için ise 0,69 olduğunu gösterdi. Duyarlılık veya özgüllük açısından istatistiksel olarak anlamlı bir fark bulunmamakla birlikte, her iki yöntemin de birbirini tamamlayıcı güçlü yönlere sahip olduğu görüldü.

**Sonuç:** MRG ve PET/BT, serviks kanserinin preoperatif değerlendirilmesinde değerlidir; MRG, lokal evrelemede üstünlük sağlarken PET/BT, lenf nodu tutulumu tespitinde daha başarılıdır. Her iki modalitenin birlikte kullanımı tanısal doğruluğu artırmaktadır. Bu bulguları doğrulamak ve klinik uygulamalar için görüntüleme stratejilerini geliştirmek adına ileriye dönük çalışmalara ihtiyaç vardır.

**Anahtar Kelimeler:** Lenf nodu, MRG, PET/BT

## Introduction

Cervical cancer ranks as the fourth most prevalent cancer among women worldwide, with 85% of cases occurring in developing countries, making it a leading cause of cancer-related deaths in women[1,2]. Data released by the Turkish Ministry of Health Cancer Department in 2025 revealed that cervical cancer had an incidence of 4.7 per 100,000, positioned it eighth among cancers affecting women in Türkiye[3]. Similarly, the American Cancer Society estimated that in 2025, 13,360 women in the United States would be diagnosed with invasive cervical cancer, leading to 4,320 deaths[4].

Persistent infection with human papillomavirus (HPV) is recognized as the primary risk factor for the development of invasive cervical cancer[5]. Although advancements in HPV vaccination and improved cervical cancer screening programs have mitigated the burden in certain regions, the disease remains a significant issue in developing countries[6].

Lymph node metastasis is a critical determinant of treatment response and overall prognosis in cervical cancer patients. Preoperative imaging methods, including positron emission tomography/computed tomography (PET/CT) and magnetic resonance imaging (MRI), play a vital role in evaluating lymph node involvement since routine surgical dissection is not always recommended[7]. The International Federation of Gynecology and Obstetrics 2018 cervical cancer staging system emphasizes the importance of pelvic and paraaortic lymph node involvement, further underscoring the necessity of preoperative imaging[8]. MRI provides detailed local staging, accurately measuring tumor size and parametrial infiltration, particularly in patients with larger tumors or during pregnancy, where radiation-free modalities are essential. Meanwhile, PET/CT offers insights into metabolic activity, aiding in the detection of distant metastases[9].

Our study aims to assess the diagnostic accuracy of preoperative PET/CT and MRI in detecting pelvic and paraaortic lymph node metastases in cervical cancer patients. We compared imaging findings with postoperative histopathological evaluations, considered the gold standard, to determine the effectiveness of these imaging modalities.

## Materials and Methods

This retrospective study reviewed cervical cancer cases diagnosed and treated at İstanbul Medeniyet University Prof. Dr. Süleyman Yalçın City Hospital's Obstetrics and Gynecology Clinic from January 1, 2016, to December 31, 2022. Inclusion criteria encompassed patients who underwent preoperative PET/CT or MRI imaging followed by pelvic and/or paraaortic lymph node dissection. Patients who lacked preoperative imaging, did not undergo lymph node sampling, or were deemed inoperable were excluded.

The patients' demographic and clinical characteristics were documented, and lymph node status based on imaging findings was compared to postoperative histopathological results.

### Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp., Armonk, NY, USA). Categorical variables were expressed as frequencies and percentages, and continuous variables were presented as means ± standard deviations. The diagnostic performance of MRI and PET/CT in detecting lymph node metastasis was evaluated by calculating sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy. Receiver operating characteristic (ROC) curve analysis was used to compare the diagnostic power of both imaging modalities, and the area under the curve (AUC) was reported. The McNemar's test was used to compare paired proportions (e.g., sensitivity and specificity) between MRI and PET/CT. A p-value of $<0.05$ was considered statistically significant.

Ethical approval for this study was granted by the Clinical Research Ethics Committee of İstanbul Medeniyet University Göztepe Training and Research Hospital under protocol number 2023/0166, dated 15.03.2023. Informed consent was not obtained due to the retrospective nature of the study, in accordance with the institutional and national ethical guidelines.

## Results

Between January 1, 2016, and December 31, 2022, a total of 75 cervical cancer cases were reviewed. Of these, 23 patients (30.6%) were excluded due to lack of preoperative imaging or being deemed inoperable. The final study cohort consisted of 52 eligible patients who met the inclusion criteria. Table 1 provides a summary of the demographic and clinical features of the study cohort.

The comparative diagnostic capabilities of MRI and PET/CT in detecting metastatic lymph nodes are outlined in Table 2. MRI exhibited a sensitivity of 55.6%, slightly higher than PET/CT's 50.0%, indicating a greater capacity than PET/CT to

identify true positive cases. However, PET/CT surpassed MRI in specificity (94.1% vs. 82.4%), showcasing better accuracy in identifying true negatives and reducing false positives.

Figure 1, depicting the ROC curves for MRI (in green) and PET/CT (in blue), demonstrates that there was no statistically significant variation in sensitivity or specificity between MRI and PET/CT, as evidenced by p-values of 1.0 and 0.26. Nevertheless, the AUC values highlighted PET/CT's superior overall diagnostic capability (with an AUC of 0.78) compared to MRI's 0.69. Table 3 summarizes previously published sensitivity and specificity values for MRI and PET/CT, offering a comparative perspective on the diagnostic performance observed in our study.

**Table 1.** Demographic and clinical characteristics of the study population

| Description | Data |
|---|---|
| Age | 50.35±10.71 |
| Smoking status | |
| Non-smokers | 31 (59.6%) |
| Smokers | 21 (40.4%) |
| BMI | 27.84±4.39 |
| Histological type | |
| Squamous cell carcinoma | 42 (80.8%) |
| Adenocarcinoma | 10 (19.9%) |
| Contraception use | |
| None | 50 (96.2%) |
| Used | 2 (3.8%) |
| HPV status | |
| Negative | 19 (36.5%) |
| Positive | 33 (63.5%) |
| Pap smear | |
| Negative | 22 (42.3%) |
| Positive | 30 (57.7%) |
| Pelvic lymph nodes | |
| Negative | 35 (67.3%) |
| Positive | 17 (32.7%) |
| Para-aortic lymph nodes | |
| Negative | 43 (82.7%) |
| Positive | 9 (17.3%) |
| Lymph nodes overall | |
| Negative | 34 (65.4%) |
| Positive | 18 (34.6%) |

**Table 2.** Performance comparison of MRI and PET/CT

| Parameter | MRI | PET/CT |
|---|---|---|
| Total, n | 52 | 52 |
| True positive, n | 10 | 9 |
| True negative, n | 28 | 32 |
| False positive, n | 6 | 2 |
| False negative, n | 8 | 9 |
| Total positive, n | 18 | 18 |
| Total negative, n | 34 | 34 |
| Sensitivity, % | 55.6 | 50 |
| Specificity, % | 82.4 | 94.1 |
| Accuracy, % | 73.1 | 78.8 |
| PPV, % | 62.5 | 81.8 |
| NPV, % | 77.8 | 78 |

PPV: Positive predictive value, NPV: Negative predictive value, PET/CT: Positron emission tomography/computed tomography, MRI: Magnetic resonance imaging

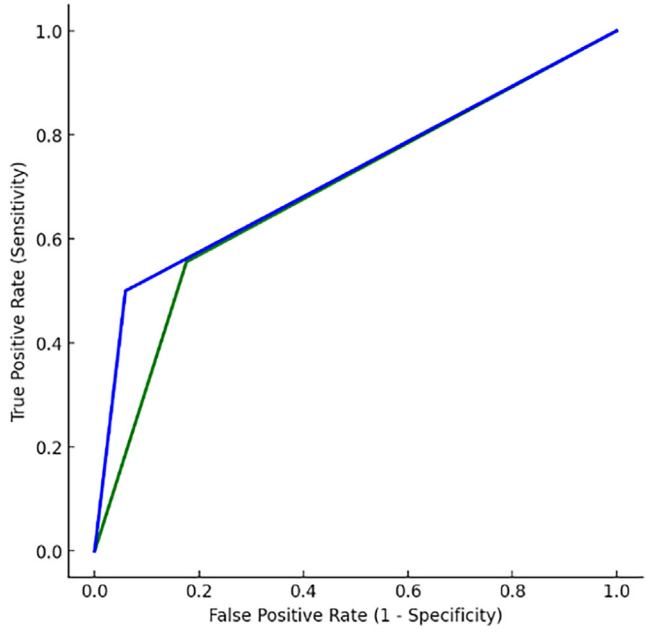


**Figure 1.** The ROC analysis comparing PET/CT and MRI

ROC curve for MRI (in green) and PET/CT (in blue), with p-values of 1.0 and 0.26 ($p<0.05$). The area under curve (AUC) of 0.78 for PET/CT shows an improvement compared to 0.69 for MRI

*PET/CT: Positron emission tomography/computed tomography, MRI: Magnetic resonance imaging, ROC: Receiver operating characteristic, AUC: Area under the curve*

**Table 3.** The sensitivity and specificity of MRI and PET/CT from the studies

| Study | Year | MRI sensitivity (%) | MRI specificity (%) | PET/CT sensitivity (%) | PET/CT specificity (%) |
|---|---|---|---|---|---|
| **Choi et al.**[10] | 2010 | 38.5 | 44.4 | 76.9 | 55.5 |
| **Chung et al.**[13] | 2010 | 64.3 | 69.1 | 28.6 | 83.6 |
| **Ferrandina et al.**[12] | 2012 | 35.7 | 95.9 | 26.8 | 97.8 |
| **Lv et al.**[15] | 2014 | 37.3 | 98.5 | 91.0 | 98.4 |
| **Jung et al.**[14] | 2017 | 24.3 | 96.3 | 48.6 | 89.3 |
| **Liu et al.**[11] | 2017 | 54.0 | 93.0 | 66.0 | 97.0 |
| **Ozen et al.** | 2025 | 55.6 | 82.4 | 50.0 | 94.0 |

PET/CT: Positron emission tomography/computed tomography, MRI: Magnetic resonance imaging

## Discussion

Accurate nodal staging is pivotal as it significantly influences both treatment planning and prognosis in cervical cancer. In this study, MRI and PET/CT demonstrated sensitivities of 55.6% and 50%, respectively, and specificities of 82.4% and 94%. These findings align with previous research, which consistently shows MRI as having higher sensitivity but lower specificity compared to PET/CT.

For pelvic lymph node involvement specifically, MRI exhibited a sensitivity of 58.8% and specificity of 82.8%, while PET/CT showed 47.1% sensitivity and 91.4% specificity. Differences in imaging performance may relate to variations in patient stages across studies. Early-stage cervical cancer tends to feature fewer metastatic lymph nodes, potentially lowering detection rates; while advanced stages often present with larger or bulkier lymph nodes, which are more readily detected.

In comparison to earlier studies, our findings were consistent. For instance, Choi et al.[10] demonstrated that PET/CT was more sensitive than MRI in identifying lymph node metastasis. Similarly, a meta-analysis by Liu et al.[11], involving 67 studies, reported higher specificity for PET/CT and greater sensitivity for MRI. Other investigations, including the studies by Ferrandina et al.[12] and Chung et al.[13], corroborated these trends, indicating that MRI performs better for local staging, while PET/CT excels in identifying distant metastasis. In their study, Jung et al.[14] investigated the comparative performance of MRI and PET/CT in detecting pelvic lymph node metastases in early-stage cervical cancer patients. In a similar context, Lv et al.[15] found PET/CT to be significantly more sensitive (91%) than MRI (31.3%) for identifying nodal metastasis in early-stage cervical cancer, emphasizing the utility of PET/CT in functional imaging.

While our results are broadly consistent with existing literature, some differences are noteworthy. Specifically, the studies by Lv et al.[15] and Jung et al.[14] found that PET/CT had greater sensitivity than MRI, whereas MRI demonstrated higher specificity-an inverse pattern compared to our findings. Several factors may account for these differences, including variability in patient characteristics, tumor staging profiles, and imaging protocols across institutions. Our cohort included a wide range of disease stages and was evaluated retrospectively in a single-center setting, potentially influencing the diagnostic outcomes. Additionally, variability in radiologic assessment and differences in imaging equipment and thresholds for interpreting tracer uptake may have contributed to the observed deviation. Table 3 provides a detailed comparison of diagnostic metrics from multiple studies.

Despite its high specificity, PET/CT is limited by moderate sensitivity, resulting in false negatives for micrometastases or small metastatic nodes. False positives also arise due to the non-specificity of the F-18 fluorodeoxyglucose tracer, which accumulates in inflamed or infected tissues[16]. MRI, with its superior soft-tissue resolution, excels in assessing tumor size and parametrial invasion, especially in early stages. However, its sensitivity in detecting nodal involvement decreases in advanced stages[17].

In our cohort, false negativity rates for nodal metastasis were 15.3% for MRI and 17.3% for PET/CT. This is consistent with the Francogyn study, which identified a false-positive rate of 15% for PET/CT and emphasized the need for careful risk stratification to mitigate these errors[18]. Additionally, a meta-analysis by Thelissen et al.[19], highlighted a 13% false-negative rate in preoperative imaging despite histopathological confirmation of lymph node metastasis postoperatively.

### Study Limitations

Among the primary constraints of this study are its retrospective methodology, the modest number of cases analyzed, and the independent assessment of radiologic and pathologic findings by different clinicians.

## Conclusion

MRI and PET/CT serve as complementary tools in the staging and management of cervical cancer. While MRI is preferred for detailed local assessments, such as tumor size and parametrial invasion, PET/CT is invaluable for evaluating lymph node involvement and detecting distant metastases. The integration

of these modalities enhances diagnostic accuracy and optimizes treatment planning. However, clinicians must remain cautious of their respective limitations, particularly regarding false positives and negatives.

Prospective studies and advancements in imaging technology are necessary to further refine diagnostic accuracy. Enhanced methods and larger patient cohorts may help address the limitations observed in retrospective analyses, ultimately improving the outcomes for cervical cancer patients.

### Ethics

**Ethics Committee Approval:** Ethical approval for this study was granted by the Clinical Research Ethics Committee of İstanbul Medeniyet University Göztepe Training and Research Hospital under protocol number 2023/0166, dated 15.03.2023.

**Informed Consent:** Informed consent was not obtained due to the retrospective nature of the study.

### Footnotes

### Authorship Contributions

Surgical and Medical Practices: S.Ö., E.D., A.T., Concept: S.Ö., Design: S.Ö., A.T., Data Collection or Processing: S.Ö., A.T., Analysis or Interpretation: S.Ö., M.S., Literature Search: S.Ö., E.D., Writing: S.Ö., M.S.

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# Effects of ovarian cyst types on ovarian reserve after three-dimensional laparoscopic cystectomy

## *Üç boyutlu laparoskopik kistektomi sonrası yumurtalık kisti tiplerinin yumurtalık rezervi üzerindeki etkileri*

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### Abstract

**Objective:** This study aims to compare the effects of three-dimensional (3D) laparoscopic ovarian cystectomy on ovarian reserve according to different types of ovarian cysts.

**Materials and Methods:** Participants who underwent surgical treatment for ovarian cysts between 2018 and 2020 were included in this study. Anti-müllerian hormone (AMH) and follicle-stimulating hormone (FSH) levels were measured before surgery and six months postoperatively. All procedures were performed under general anesthesia using 3D laparoscopy. Participants were classified into three groups based on histopathological findings: group 1, endometriomas; group 2, mature cystic teratomas (dermoid cysts); and group 3, serous or mucinous cystadenomas.

**Results:** A total of 51 women were included in the study. No significant differences were observed between the groups in terms of perioperative variables such as operation time, intraoperative blood loss, postoperative hemoglobin decrease, and maximum cyst diameter. There were also no significant differences among the groups in preoperative AMH (p=0.97) and FSH (p=0.22) levels. Postoperative AMH levels were significantly lower than preoperative values in both the endometrioma group (p<0.001) and the dermoid cyst group (p=0.004). The reduction in AMH levels was more pronounced in the endometrioma group compared to the other groups. Postoperative FSH levels tended to increase in all groups compared to preoperative levels; however, this increase was not statistically significant (p=0.092).

**Conclusion:** 3D laparoscopic cystectomy for the removal of endometriomas and dermoid cysts significantly reduces ovarian reserve. In contrast, laparoscopic cystectomy for serous or mucinous cysts appears to have no significant impact on ovarian reserve.

**Keywords:** 3D laparoscopy, AMH, dermoid cyst, endometrioma ovarian cyst, ovarian reserve

### Öz

**Amaç:** Çalışmamızın amacı, farklı kist tipleri açısından üç boyutlu (3D) laparoskopik over kistektomisinin over rezervi üzerindeki etkisini karşılaştırmaktır.

**Gereç ve Yöntemler:** Çalışmamıza 2018-2020 yılları arasında over kistleri nedeniyle cerrahi tedavi görecek katılımcılar dahil edildi. Katılımcıların anti-müller hormon (AMH) ve folikül uyarıcı hormon (FSH) düzeyleri ameliyat öncesi ve ameliyattan 6 ay sonra ölçüldü. Tüm ameliyatlar genel anestezi altında ve 3D laparoskopi ile gerçekleştirildi. Histopatolojik değerlendirmeden sonra katılımcılar üç gruba ayrıldı; grup 1: Endometrioma, grup 2: Olgun kistik teratom (dermoid kist) ve grup 3: Seröz veya müsinöz kistadenom.

**PRECIS:** Three-dimensional laparoscopic cystectomy significantly reduces ovarian reserve in endometrioma and dermoid cysts but not in serous-mucinous cysts, highlighting cyst-type-specific impacts on ovarian function.

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**Bulgular:** Çalışmaya toplam 51 kadın dahil edildi. Gruplar arasında ameliyat süresi, intraoperatif kan kaybı, postoperatif hemoglobin azalması ve maksimum kist çapı gibi perioperatif bulgular açısından anlamlı bir fark bulunmadı. Gruplar arasında preoperatif AMH (p=0,97) ve preoperatif FSH (p=0,22) değerleri açısından anlamlı bir fark yoktu. Endometrioma (p<0,001) ve dermoid kist (p=0,004) gruplarında postoperatif AMH değerleri preoperatif AMH değerlerine kıyasla anlamlı derecede düşüktü. Preoperatif ve postoperatif AMH azalması endometriozis grubunda diğer gruplara göre daha belirgindi. Tüm gruplardaki postoperatif FSH seviyeleri preoperatif seviyelere kıyasla artma eğilimindeydi, ancak artış anlamlı bir seviyeye ulaşmadı (p=0,092).

**Sonuç:** Endometrioma ve dermoid kistleri çıkarmak için yapılan 3D laparoskopik kistektomi over rezervini önemli ölçüde azaltır. Seröz-müsinöz kistler için yapılan laparoskopik kistektomi over rezervi etkilemez.

**Anahtar Kelimeler:** 3D laparoskopi, AMH, dermoid kist, endometrioma, over kisti, over rezervi

## Introduction

Ovarian cysts affect approximately 7% of women during their lifetime and are particularly common during the reproductive years[1]. Given that ovarian cysts often become symptomatic during the reproductive years, fertility preservation is a primary concern in their management. For clinicians, this concern is typically assessed through evaluation of ovarian reserve (OR).

While age remains the leading factor influencing OR, medical interventions like surgery, radiation, and chemotherapy can also have a negative impact on its status[2]. Several biochemical and ultrasonographic markers are used to assess OR. Among these, anti-müllerian hormone (AMH) stands out as a reliable and practical indicator[3]. Moreover, AMH serves as a sensitive marker for assessing iatrogenic damage to ovarian function[4]. In addition, the measurement of basal follicle-stimulating hormone (FSH) levels is a well-established, straightforward, and reliable method for assessing OR[5]. FSH is a glycoprotein polypeptide hormone produced by the gonadotropic cells of the anterior pituitary gland[6].

Although simple functional cysts are typically managed conservatively to preserve OR during reproductive age, surgical intervention is often indicated for cyst types such as endometriomas and mature cystic teratomas, which are unlikely to resolve spontaneously, particularly in the presence of suspicious ultrasonographic findings.

Laparoscopic ovarian cystectomy is the preferred surgical method for ovarian cysts suspected to be benign. Although this procedure is typically regarded as fertility-preserving, recent concerns have arisen about its possible effects on OR. During cyst enucleation, there is a risk of unintentionally removing healthy ovarian tissue or causing mechanical or thermal damage to the ovarian cortex[1]. Literature presents conflicting evidence, and no definitive conclusions have been reached regarding this issue.

Another unresolved question in the literature concerns the comparative advantages of two-dimensional (2D) versus three-dimensional (3D) laparoscopy. 3D laparoscopy offers enhanced depth perception and spatial orientation compared to conventional 2D laparoscopy, potentially enabling greater surgical precision. This improved precision may enhance the surgeon's performance during laparoscopic ovarian procedures and help mitigate the negative impact on OR. Several studies have demonstrated the specific advantages of 3D laparoscopy, particularly during the dissection and laparoscopic suturing phases[7].

This study was designed to evaluate the impact of cystectomy performed using 3D laparoscopy on OR across different cyst types, with the aim of contributing to the clinical management of such cases.

## Materials and Methods

Patients who presented to the Department of Obstetrics and Gynecology at Ege University Faculty of Medicine between 2018 and 2020, and underwent surgical treatment for ovarian cysts were evaluated for inclusion in this prospective cohort study based on predefined inclusion and exclusion criteria. All participants were informed about the study both verbally and in writing, and written informed consent was obtained prior to enrollment. The study protocol was approved by the Ethics Committee of Ege University Faculty of Medicine on April 29, 2016 (approval number: 16-4T/56). This research was supported by the Ege University Scientific Research Projects Coordination Unit under project number 17-TIP-056. All procedures in this study were carried out in compliance with the ethical guidelines established by the institutional research committee, the 1964 Declaration of Helsinki, and its subsequent revisions or equivalent ethical standards. The study was registered at ClinicalTrials.gov (NCT05054946).

The inclusion criteria were defined as being between 18 and 40 years of age and having no history of prior ovarian surgery, to standardize the cohort and eliminate potential iatrogenic effects on OR. Exclusion criteria consisted of the presence of endocrinological disorders, suspected malignancy based on preoperative laboratory and ultrasonographic assessments, or histopathological confirmation of malignancy, as these factors could influence OR. The main outcome measure was serum AMH levels, which were evaluated six months after surgery.

All participants underwent a preoperative evaluation of cyst size and morphology using either transvaginal or transabdominal ultrasonography (Voluson E8, GE Healthcare, Chicago, IL, USA) employing a RAB 4-8D 4D transabdominal probe and a multifrequency 5-7.5 MHz transvaginal probe. Cyst size was calculated by averaging the maximum diameter and the diameter perpendicular to it as measured during ultrasonographic examination. Blood samples were collected from all participants during the early follicular phase (days 1-5 of the menstrual cycle) following 8-10 hours of fasting. The

same procedure was repeated for six months postoperatively. To measure serum AMH concentrations, blood samples were first centrifuged at 1600 g for 10 minutes. The separated serum fractions were then preserved at -80 °C until the completion of sample collection from all participants. Subsequently, AMH levels were analyzed in batches using the AMH Gen II ELISA kit provided by Beckman Coulter Inc. (Brea, CA, USA). For FSH measurement, blood samples were collected in separator gel tubes, centrifuged at 1400 g for 10 minutes, and analyzed using the Beckman Coulter DXI 800 immunoassay system (Beckman Coulter Inc., Brea, CA, USA).

Based on histopathological findings, the participants were classified into three distinct groups: Group 1 included cases of endometrioma, group 2 comprised mature cystic teratomas (dermoid cysts), and group 3 encompassed serous or mucinous cystadenomas. During surgery, the cyst wall was carefully dissected and stripped from the adjacent normal ovarian tissue. Hemostasis was achieved by applying bipolar coagulation at bleeding sites. When required, ovarian reconstruction was performed using a 4-0 absorbable suture (polyglycolic acid, Vicryl Rapide™, Ethicon, Germany). To minimize the risk of postoperative adhesion, the surgical field was irrigated extensively with warm saline. All participants were discharged on the first postoperative day following an uneventful recovery period.

### Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 22.0 (IBM Corp., Armonk, NY, USA). The distribution of variables was examined through the Kolmogorov-Smirnov test. For continuous variables, descriptive statistics are presented as mean ± standard deviation for normally distributed data and as median (range) for non-normally distributed data. Categorical variables are represented as frequencies and percentages [n (%)]. The chi-square test or Fisher's exact test was used for comparing categorical variables, as appropriate. For continuous variables, the Student's t-test was applied when the data followed a normal distribution, while the Mann-Whitney U test was used for non-normally distributed data. A p-value of <0.05 was considered statistically significant.

## Results

According to the inclusion criteria, 51 participants were initially enrolled in the study. However, four participants with hypothyroidism, three with malignancies identified through frozen section and final histopathological evaluation, and one who failed to attend the 6-month follow-up were subsequently excluded. No participants declined to participate in the study. Consequently, a total of 43 participants were included in the final analysis.

The mean age of the participants was 28.19±6.14 years in the endometrioma group (n=16), 28.38±6.70 years in the dermoid cyst group, and 26.82±5.72 years in the serous or mucinous cystadenoma group. No significant differences were observed between the groups regarding age, gravidity, or parity (Table 1).

The mean operative times were 68.13±21.86 minutes in the endometrioma group, 74.38±28.10 minutes in the dermoid cyst group, and 77.73±25.53 minutes in the serous/mucinous cyst group. There were no significant differences between the groups (p=0.56) (Table 2). Intraoperative blood loss and postoperative hemoglobin decrease were as follows: 83.75±95.57 mL and 1.55±0.78 g/dL in the endometrioma group; 35.00±35.37 mL and 1.56±1.03 g/dL in the dermoid cyst group; and 71.09±58.00 mL and 1.42±0.77 g/dL in the serous/mucinous cyst group. There were no statistically significant differences among the groups in terms of intraoperative blood loss (p=0.054) or postoperative hemoglobin reduction (p=0.9) (Table 2).

The mean maximum cyst diameters were 54.65±14.91 mm in the endometrioma group, 51.68±16.16 mm in the dermoid cyst group, and 56.09±8.75 mm in the serous/mucinous cyst group. No significant differences were found between the groups regarding cyst diameter (p=0.7) (Table 2).

Preoperative AMH levels were 4.20±3.27 ng/mL in the endometrioma group, 4.40±4.28 ng/mL in the dermoid cyst group, and 4.14±3.06 ng/mL in the serous/mucinous cyst group. The mean preoperative FSH levels were 6.56±2.58 mIU/mL, 6.51±2.46 mIU/mL, and 7.34±0.98 mIU/mL in the endometrioma, dermoid cyst, and serous/mucinous cyst groups, respectively. There were no significant differences among the groups in terms of preoperative AMH (p=0.97) or FSH levels (p=0.22) (Table 3).

**Table 1.** Patients' characteristics

| | Endometrioma (n=16) | | Dermoid cyst (n=16) | | Serous or mucinous cystadenoma (n=11) | | p |
|---|---|---|---|---|---|---|---|
| | Mean SD | Median (Min-Max) | Mean SD | Median (Min-Max) | Mean SD | Median (Min-Max) | |
| **Age (year)** | 28.19±6.14 | 27 (19-39) | 28.38±6.70 | 29 (18-40) | 26.82±5.72 | 25 (20-37) | 0.79 |
| **Gravida** | 0.44±1.0 | 0 (0-4) | 0.94±1.28 | 0 (0-4) | 0.45±0.93 | 0 (0-3) | 0.37 |
| **Parity** | 0.38±0.80 | 0 (0-3) | 0.50±0.73 | 0 (0-2) | 0.36±0.67 | 0 (0-2) | 0.73 |

SD: Standard deviation, Min: Minimum, Max: Maximum

Group-wise analysis revealed a significant postoperative decline in AMH levels compared to preoperative values in both the endometrioma group (p<0.001) and the dermoid cyst group (p=0.004). In contrast, the serous/mucinous cyst group did not exhibit a statistically significant change in AMH levels postoperatively (p=0.297). The reduction in AMH was more pronounced in the endometrioma and dermoid cyst groups compared to the serous/mucinous cyst group (Figure 1). However, no significant difference was found between the endometrioma and dermoid cyst groups in terms of AMH reduction.

Comparison of preoperative and postoperative FSH levels showed a trend toward increased postoperative FSH values across all three groups; however, this increase did not reach statistical significance (p=0.092) (Figure 2).

No significant differences were observed among the three groups regarding postoperative AMH (p=0.14) and FSH (p=0.15) levels.

**Table 2.** Peri-operative findings

| | Endometrioma (n=16) | | Dermoid cyst (n=16) | | Serous or mucinous cystadenoma (n=11) | | p |
|---|---|---|---|---|---|---|---|
| | Mean SD | Median (Min-Max) | Mean SD | Median (Min-Max) | Mean SD | Median (Min-Max) | |
| **Surgery time (min)** | 68.13±21.86 | 61 (45-110) | 74.38±28.10 | 70 (35-130) | 77.73±25.53 | 70 (40-120) | 0.56 |
| **Blood loss (mL)** | 83.75±95.57 | 52.50 (10-400) | 35.00±35.37 | 22.50 (2-110) | 71.09±58.00 | 50 (10-190) | 0.054 |
| **Hb change** | 1.55±0.78 | 1.45 (0.60-3.10) | 1.56±1.03 | 1.30 (0.50-4.20) | 1.42±0.77 | 1.20 (0.50-2.50) | 0.9 |
| **Max cyst diameter (mm)** | 54.65±14.91 | 51 (34-81) | 51.68±16.16 | 52.75 (23.5-74) | 56.09±8.75 | 55 (40-75.5) | 0.70 |

SD: Standard deviation, Min: Minimum, Max: Maximum, Hb: Hemoglobin, min: Minutes

**Table 3.** Ovarian reserve markers

| | Endometrioma (n=16) | | Dermoid cyst (n=16) | | Serous or mucinous cystadenoma (n=11) | | p |
|---|---|---|---|---|---|---|---|
| | Mean SD | Median (Min-Max) | Mean SD | Median (Min-Max) | Mean SD | Median (Min-Max) | |
| Preop AMH (ng/mL) | 4.20±3.27 | 2.76 (0.67-10.94) | 4.40±4.28 | 2.52 (0.81-16.12) | 4.14±3.06 | 4.09 (0.67-9.42) | 0.97 |
| Postop AMH (ng/mL) | 2.14±2.24 | 1.59 (0.15-7.84) | 3.16±2.88 | 2.27 (0.50-11.8) | 3.63±3.24 | 2.49 (0.92-11.81) | 0.14 |
| Preop FSH (mlU/mL) | 6.56±2.58 | 7.14 (0.30-10.09) | 6.51±2.46 | 6.56 (1.68-10.77) | 7.34±0.98 | 7.08 (6.31-9.89) | 0.22 |
| Postop FSH (mlU/mL) | 7.67±3.51 | 6.78 (2.94-16.66) | 7.12±3.43 | 7.30 (1.45-16.41) | 8.33±1.31 | 8.13 (5.65-10.4) | 0.15 |

SD: Standard deviation, Min: Minimum, Max: Maximum, AMH: Anti-müllerian hormone, FSH: Follicle-stimulating hormone, Preop: Preoperative, Postop: Postoperative

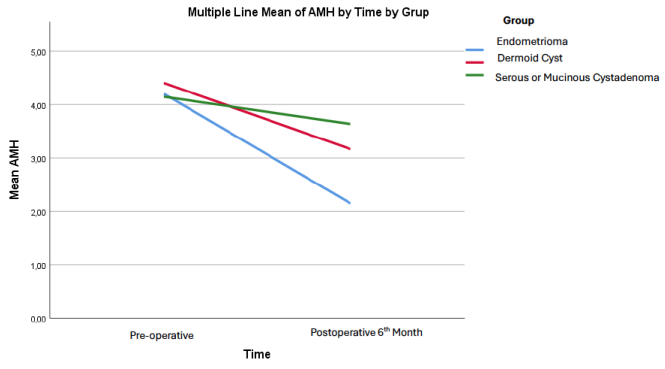


**Figure 1.** xx

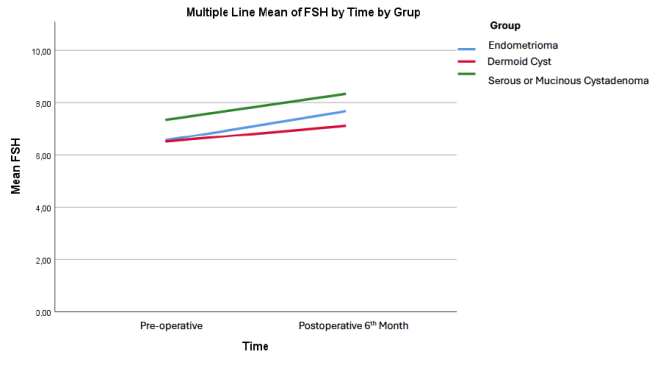


**Figure 2.** xx

## Discussion

In this prospective cohort study, the impact of 3D laparoscopic ovarian cystectomy on OR was evaluated with respect to varying types of ovarian cysts. The analysis demonstrated no statistically significant differences in preoperative AMH (p=0.97) or FSH (p=0.22) levels among the groups, suggesting that the presence of endometriomas, dermoid cysts, or serous/mucinous cysts does not independently influence baseline OR. However, by the end of the study, surgical intervention for endometriomas and dermoid cysts was associated with a significant decline in OR, whereas no such impact was observed in patients with serous or mucinous cysts.

Endometrioma is defined as the presence of ectopic endometrial tissue within the ovary. Surgical treatment is often indicated to alleviate pelvic pain, prevent complications such as cyst rupture or ovarian torsion, exclude malignancy, and address infertility. Among its potential complications are ovarian torsion and spontaneous rupture. In subfertile patients, surgical removal of endometriomas has been associated with improved pregnancy rates[8]. While surgical treatment of endometriomas has been shown to increase spontaneous pregnancy rates, it does not appear to significantly influence the outcomes of in vitro fertilization[8]. Whether endometriomas impair ovarian function remains a subject of ongoing debate. In a study evaluating 1,199 cycles in 244 women with unilateral endometrioma, ovulation was found to occur at similar rates in both the affected and unaffected ovaries[9]. In contrast, a meta-analysis encompassing eight studies demonstrated that endometrioma excision led to an average reduction of 38% in serum AMH levels, suggesting a potentially detrimental effect on OR[10]. OR loss is higher in recurrent surgeries compared to one definitive surgery[11]. Therefore, repetitive ovarian endometrioma surgeries should be avoided as much as possible. The recurrence rate of endometriomas is up to 25%[12]. Postoperative hormonal suppression options should be considered to avoid repetitive surgeries.

Ovarian germ cell tumors arise from ovarian primordial germ cells and constitute 20-25% of ovarian neoplasms. Malignancies are detected in only 5% of these neoplasms[13]. These tumors usually affect females between the ages of 10 to 30. Benign cystic mature teratomas, also known as dermoid cysts, are the most frequently encountered ovarian germ cell tumors. Almost all are benign[14]. Surgery is recommended for confirmation of diagnosis, exclusion of malignancy, preservation of OR, prevention of complications such as torsion and rupture, and relieving symptoms, if any. The abdomen should be washed with plenty of water to avoid chemical peritonitis at the end of the surgery.

Serous and mucinous cystadenomas are the most common benign ovarian neoplasms. These cysts may be thin-walled, unilocular, or multilocular, and typically range in size from 5 to 20 cm. Mucinous cysts are less common than serous cysts and are more frequently multilocular. Histologically, the inner lining of serous cysts resembles tubal epithelium, whereas mucinous cysts are usually lined with endocervical or gastrointestinal-type epithelium. Most serous and mucinous cystadenomas are asymptomatic and are often detected incidentally during ultrasound examinations.

According to current literature, surgical excision of endometriomas is generally associated with a more substantial decline in OR compared to other cyst types. The impact of dermoid cyst removal on OR remains controversial. One study investigating the early postoperative effects of laparoscopic unilateral ovarian cystectomy reported comparable reductions in AMH levels in both the endometrioma and dermoid cyst groups, with significantly smaller decreases observed in the simple cyst group[15]. Although our study focuses on longer-term outcomes, these findings align with our observations.

Cyst size also plays a role in OR outcomes. Henes et al.[16] demonstrated a significant AMH decrease post-surgery only in women with cysts ≥5 cm. Conversely, Lind et al.[1] reported that factors such as cyst size, patient age, timing of surgery, and intraoperative blood loss did not significantly influence OR. In their study, which included 75 women undergoing ovarian surgery, AMH levels were measured preoperatively and at six months postoperatively. A significant reduction in AMH levels was observed in patients with endometriomas when compared to those with dermoid cysts. While a postoperative decrease in AMH was also noted in the functional cyst group, it did not reach statistical significance. Interestingly, the study revealed that patients with elevated baseline AMH levels (AMH >4 ng/mL) experienced a more substantial decrease in AMH six months after surgery compared to those with normal or low baseline AMH levels[1]. Our findings are consistent with previous research, demonstrating a greater decline in OR in the endometrioma group compared to the dermoid cyst group. However, this difference did not reach statistical significance.

In another prospective observational study involving 71 women undergoing their first unilateral ovarian cystectomy via laparoscopy, investigators assessed antral follicle count, ovarian volume, resistance index, and OR at six months postoperatively. The aim was to evaluate the impact of different cyst types on OR by comparing the operated ovary with the contralateral one. The study concluded that surgical intervention led to a reduction in OR, regardless of cyst type[17]. This study is inconsistent with many studies in the literature and our study.

A retrospective evaluation of 97 patients revealed that postoperative AMH levels declined more markedly in individuals with endometrioma than in those with other types of benign ovarian cysts. Furthermore, an inverse correlation was identified between cyst size and preoperative AMH concentrations[18].

The reduction in OR following endometrioma excision is primarily attributed to the unintentional removal of healthy

ovarian tissue, which may occur due to the invaginating nature of the cyst into the ovarian cortex[19]. While the reduction in OR following endometrioma excision can be explained by the mechanisms described above; the decline associated with dermoid cyst removal is thought to result from the cyst's firm adhesion to the ovarian tissue and the hemostatic techniques employed during surgery[1]. Conversely, the reduction in OR during dermoid cyst removal is often attributed to cyst adherence and the hemostatic methods employed.

3D laparoscopy has been reported to provide significant benefits in complex surgical procedures, including improved image quality, reduced operative time, and decreased intraoperative blood loss. Despite these advancements, particularly enhanced depth perception and greater surgical precision, our study demonstrated that the use of 3D laparoscopy in ovarian surgery did not prevent the decline in OR associated with different cyst types[20,21]. While we observed significant postoperative AMH reductions in the endometrioma and dermoid cyst groups, FSH levels did not show a similar pattern, possibly due to differing kinetics during the menstrual cycle compared to AMH. The findings of our study are further substantiated by the recent systematic reviews and meta-analyses conducted by Moreno-Sepulveda et al.[22] and Samartzis et al.[23], both of which emphasize the limited efficacy of current surgical techniques in preserving OR during endometrioma surgery. Their conclusions resonate with our observations, reinforcing the notion that despite advancements in surgical approaches, the preservation of ovarian function remains a significant clinical challenge.

### Study Limitations

Our study's strengths include its prospective design, robust sample size across various cyst types, and standardized surgical protocols by experienced minimally invasive surgeons at a tertiary university hospital. Future studies could explore additional factors influencing OR outcomes post-ovarian cystectomy, including long-term hormonal assessments and fertility outcomes.

## Conclusion

This study demonstrates that 3D laparoscopic cystectomy for endometriomas and dermoid cysts is associated with a significant reduction in OR, whereas cystectomy for serous and mucinous cysts does not appear to have a significant impact. Preoperative assessment of OR and appropriate patient counseling regarding potential fertility implications are particularly important for reproductive-aged women undergoing ovarian cyst surgery.

### Ethics

**Ethics Committee Approval:** The study protocol was approved by the Ethics Committee of Ege University Faculty of Medicine on April 29, 2016 (approval number: 16-4T/56).

**Informed Consent:** Written informed consent was obtained prior to enrollment.

### Footnotes

### Authorship Contributions

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

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

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# Unexpected hypo-responders in in vitro fertilization: The impact of higher gonadotropin doses on oocyte yield

## *Tüp bebekte beklenmedik düşük yanıt veren grupta: Daha yüksek gonadotropin dozlarının oosit verimine etkisi*

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### Abstract

**Objective:** This study assessed the impact of increased initial gonadotropin doses on ovarian stimulation (OS) outcomes in unexpected hypo-responders [Patient-Oriented Strategies Encompassing Individualized Oocyte Number (POSEIDON) 1-2 group] with suboptimal mature oocyte yield, despite normal ovarian reserve markers, during their first OS cycle.

**Materials and Methods:** Conducted at a referral infertility clinic, this observational study included women who retrieved fewer than nine oocytes during their first OS cycle despite gonadotropin doses of 150-225 international unit (IU)/day starting from cycle day two. Women who underwent a second OS cycle following unsuccessful conception were included. Gonadotropin doses were increased to 225 or 300 IU recombinant follicle-stimulating hormone (FSH) (recFSH) based on body mass index. Each patient served as her own control, with first and second OS cycles compared in terms of oocyte yield, follicular output ratio (FORT), and follicle-to-oocyte index (FOI).

**Results:** Among 289 unexpected hypo-responders (12% prevalence), the mean age was 34.2 years, and the mean anti-müllerian hormone level was 3.4 ng/mL. The stimulation duration was similar between cycles (11.2 days). The second OS cycle showed significant improvements in total oocytes, metaphase II oocytes, FORT, FOI, cleavage-stage embryos, and blastocysts ($p<0.05$).

**Conclusion:** Increasing gonadotropin doses in subsequent cycles improves oocyte yield and embryological outcomes in unexpected hypo-responders (POSEIDON 1-2) with normal ovarian reserve markers.

**Keywords:** Infertility, ovulation induction, *in vitro* oocyte maturation techniques

### Öz

**Amaç:** Gonadotropin başlangıç dozlarının artırılması, normal over rezervi belirteçlerine rağmen suboptimal yanıt gösteren beklenmedik düşük yanıtlayıcılar [Bireyselleştirilmiş Oosit Sayısını Kapsayan Hasta Odaklı Stratejiler (POSEIDON) 1-2] için oosit verimini ve embriyolojik sonuçları iyileştirir. Bu çalışma, ilk over stimülasyon (OS) sikluslarında normal over rezerv belirteçlerine rağmen yetersiz olgun oosit verimi gösteren beklenmedik düşük yanıt verenlerde (POSEIDON 1-2 grubu) artan gonadotropin başlangıç dozlarının OS sonuçları üzerindeki etkisini değerlendirdi.

**Gereç ve Yöntemler:** Bir referans infertilite kliniğinde yürütülen bu gözlemsel çalışmaya, ilk OS sikluslarında günlük 150-225 uluslararası birim (IU) gonadotropin dozları almasına rağmen dokuzdan az oosit toplayan kadınlar dahil edildi. Gebelik elde edemeyip ikinci bir OS siklusuna geçen kadınlar

**PRECIS:** Increasing gonadotropin starting doses improves oocyte yield and embryological outcomes in unexpected hypo-responders (POSEIDON 1-2) with suboptimal response, despite normal ovarian reserve markers.

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çalışmaya alındı. Vücut kitle indeksine göre gonadotropin başlangıç dozları 225 veya 300 IU rekombinant folikül uyarıcı hormon (FSH) (recFSH) olarak artırıldı. Her hasta, kendi tarihi kontrolü olarak değerlendirilerek, ilk ve ikinci OS siklusları karşılaştırıldı. Karşılaştırma, oosit verimi, foliküler çıktı oranı (FORT) ve folikül-oosit indeksi (FOI) üzerine odaklandı.

**Bulgular:** Çalışma süresince beklenmedik düşük yanıtlayıcı (POSEIDON 1-2) olarak tanımlanan toplam 289 kadın (%12 prevalans) belirlendi. Ortalama yaş 34,2 yıl ve ortalama anti-müllerian hormon seviyesi 3,4 ng/mL idi. Stimülasyon süresi her iki siklus arasında benzerdi (11,2 gün). Ancak ikinci OS siklusunda toplam oosit, metafaz II oosit, FORT, FOI, klivaj evresi embriyoları ve blastosist sayılarında anlamlı iyileşmeler gözlendi (p<0,05).

**Sonuç:** Sonraki sikluslarda gonadotropin dozlarının artırılması, normal over rezerv belirteçlerine sahip beklenmedik düşük yanıtlayıcılar (POSEIDON 1-2) için oosit verimini ve embriyolojik sonuçları iyileştirmektedir.

**Anahtar Kelimeler:** İnfertilite, ovülasyon indüksiyonu, *in vitro* oosit maturasyon teknikleri

## Introduction

Ovarian stimulation (OS) is a cornerstone of assisted reproductive technology (ART), designed to recruit multiple follicles and obtain an adequate number of mature oocytes for embryo development. The number of mature oocytes retrieved is a key determinant of ovarian response to exogenous gonadotropins and is strongly correlated with cumulative live birth rates across fresh and frozen embryo transfer cycles[1].

Despite having normal ovarian reserve markers, some patients exhibit an unexpected hypo-response to OS, characterized by a suboptimal oocyte yield (<9) in their first cycle. This phenomenon has important implications for reproductive outcomes, as lower oocyte yields are associated with fewer available embryos, decreased cumulative live birth rates, and increased emotional and financial burdens for patients. While this condition is defined within the Patient-Oriented Strategies Encompassing Individualized Oocyte Number (POSEIDON) classification system, unexpected hypo responders constitute a distinct and increasingly recognized subgroup requiring targeted interventions to optimize ART success[2,3].

To assess unexpected hypo response, markers such as the follicular output rate (FORT) and follicle-to-oocyte index (FOI) provide valuable insights. These parameters evaluate the relationship between antral follicle count (AFC) and the number of retrieved oocytes, offering a more qualitative measure of ovarian responsiveness[4]. Patients with FORT or FOI values below 50% are typically classified as hypo-responders, highlighting the discrepancy between expected and actual ovarian response.

Determining the optimal gonadotropin dose for this subgroup is crucial for enhancing follicular recruitment, oocyte yield, and subsequent ART outcomes[5]. This study aims to investigate the impact of increasing the initial gonadotropin dose in patients who exhibited an unexpected hypo-response during their first OS cycle. By evaluating oocyte yield and secondary markers such as FORT and FOI in subsequent cycles, we aim to provide evidence-based guidance for optimizing stimulation protocols in this challenging patient population.

## Materials and Methods

This observational study was conducted at a referral in vitro fertilization (IVF) center between October 2022 and September 2023. The study adhered to the principles of the Helsinki Declaration and was approved by the Üsküdar University Faculty of Medicine Ethics Committee on October 26, 2020 (protocol number: 61351342/OCTOBER 2022-24). Written informed consent was obtained from all participants.

Eligible patients included infertile women who exhibited an unexpected hypo response during their first OS cycle, defined as the retrieval of fewer than nine oocytes despite normal ovarian reserve tests (ORTs). Normal ORT was defined as serum anti-müllerian hormone (AMH) levels between 1-4 ng/mL and an AFC of 5-12 in both ovaries. The standard policy at the IVF center for initial gonadotropin dosing in patients with normal ORTs involved starting recombinant follicle-stimulating hormone (FSH) (recFSH) at 150 international unit (IU)/day for women with a body mass index (BMI) of 20-25 kg/m$^2$ or 225 IU/day for women with a BMI >25 kg/m$^2$[6-8].

Patients who underwent a second OS cycle within 12 months of the first cycle due to failure to conceive were included in this analysis. In the second OS cycle, a uniform dose escalation strategy was implemented to all patients. As a result, women with a BMI of 20-25 kg/m$^2$ received 225 IU/day of recFSH, while those with a BMI >25 kg/m$^2$ received 300 IU/day, beginning on the second day of the menstrual cycle. Each patient's first cycle served as a historical control for comparison of outcomes.

Exclusion criteria included patients with diminished ovarian reserve markers; failed follicular response during OS; presumed risk of ovarian hyperstimulation syndrome (total of >20 follicles measuring >12 mm on the day of hCG); inability to proceed with the second OS cycle; use of OS protocols other than GnRH antagonists; use of urinary gonadotropins; OS cycles involving dual ovulation triggering; preimplantation genetic testing for aneuploidy cycles; elevated serum progesterone (P) levels on the day of hCG administration; or an interval of more than 12 months between the two oocyte retrieval cycles.

The primary outcomes of this study were the FORT and FOI[9]. Secondary outcomes included the number of metaphase II (M2) oocytes retrieved and the number of good-quality embryos available.

OS was initiated using recFSH (Gonal-F, Merck Serono, Istanbul) on the second day of the menstrual cycle, without any pre-treatment such as oral contraceptives. A GnRH antagonist (Cetrotide, Merck Serono, Istanbul; 0.25 mg/day) was introduced on the sixth day of stimulation and continued

throughout the cycle. Final oocyte maturation was triggered with recombinant human chorionic gonadotropin (rhCG) (250 mcg; Ovitrelle, Merck Serono, Istanbul) when at least three follicles reached ≥18 mm in diameter. Transvaginal ultrasound-guided oocyte retrieval and subsequent embryo transfer procedures were conducted as previously described[10,11].

## Results

During the study period, 289 women were identified as unexpected hypo responders based on their first OS cycle within a cohort of 2,390 infertile women, representing an overall prevalence of 12% (Figure 1). The median age of the participants was 34.00 years (24-42), with a median AMH level of 2.60 ng/mL (1.28-16.20) and an infertility duration of 5.00 years (1.00-22.00). Among these, 103 women (35.6%) underwent a second OS cycle, with a mean interval of 8.4 months between cycles. Demographic and clinical characteristics are summarized in Table 1.

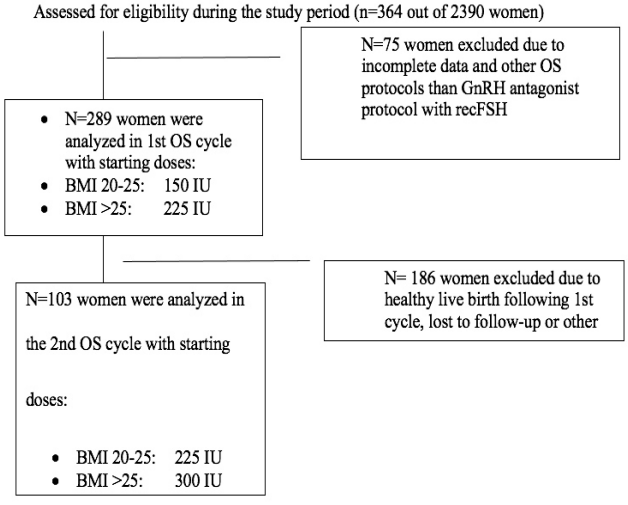


**Figure 1.** Flowchart of the study

*OS: Oocyte stimulation, BMI: Body mass index, recFSH: Recombinant follicle-stimulating hormone, IU: International unit*

Patients in the tailored gonadotropin dose group received significantly higher starting and total gonadotropin doses compared to the standard group (p<0.0001), while the stimulation duration remained comparable (p=0.26). Key ovarian response markers improved significantly, with higher median FORT (0.69 *vs.* 0.44, p<0.0001), FOI (0.67 *vs.* 0.38, p<0.0001), and total oocytes retrieved (9.00 *vs.* 5.00, p<0.0001) (Figures 2, 3).

Enhanced embryological outcomes were observed in the tailored dose group, including higher numbers of day 3 embryos (5.00 *vs.* 2.00, p<0.0001), day 5 embryos (3.00 *vs.* 2.00, p<0.0001), and top-quality blastocysts (2.00 *vs.* 0.00, p<0.0001), highlighting the benefits of dose escalation (Table 2, Table 3).

## Discussion

This study demonstrates that tailored gonadotropin dose adjustments in unexpected hypo responders significantly improve oocyte yield, follicular response markers (FORT and FOI), and embryological outcomes, highlighting the importance of individualized OS protocols in optimizing ART success[5,6]. There is limited data on outcomes in POSEIDON groups 1b and 2b within Türkiye. A recent retrospective study

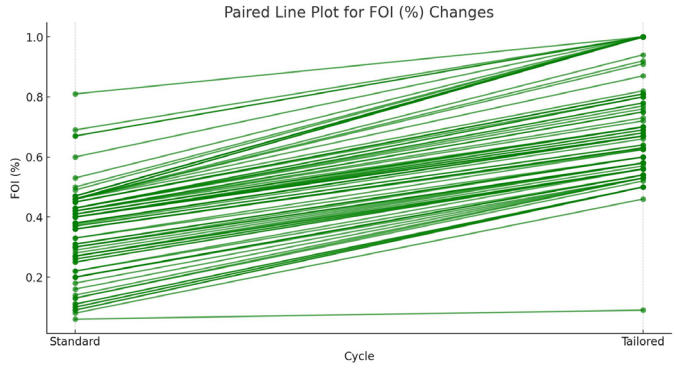


**Figure 2.** Paired line plot for FOI (%) changes

*FOI: Follicle oocyte index*

**Table 1.** Demographic data of standard and tailored groups

| Parameter | Stat type | Standard group | Tailored group | p-value |
|---|---|---|---|---|
| Age (years) | Median (minimum, maximum) | 34.00 (24.00, 42.00) | 34.00 (24.00, 42.00) | 0.982 |
| Cause of infertility | 1: 23% (unexplained)<br>2: 27% (male factor)<br>3: 16% (anovulatory)<br>4: 34% (other) | | | |
| Duration of infertility (years) | Median (minimum, maximum) | 5.00 (1.00, 22.00) | 6.00 (1.00, 22.00) | 0.314 |
| BMI (kg/m$^2$) | Median (minimum, maximum) | 22.00 (18.00, 30.00) | 22.00 (18.00, 30.00) | 0.994 |
| AFC (N) | Median (minimum, maximum) | 11.00 (6.00, 19.00) | 11 (6.00, 19.00) | 0.580 |
| AMH (ng/mL) | Median (minimum, maximum) | 2.60 (1.28, 16.20) | 2.71 (1.28, 16.20) | 0.570 |

BMI: Body mass index, AFC: Antral follicle count, AMH: Anti-müllerian hormone

reported significantly lower live birth rates in this subgroup compared to normal responders, emphasizing the need for more personalized treatment strategies[7].

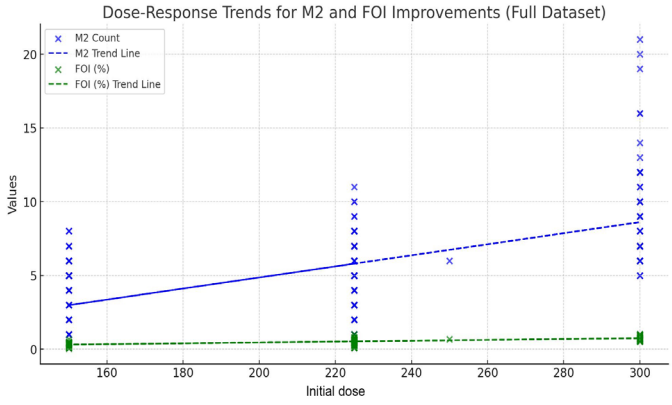


**Figure 3.** Dose-response trends for M2 and FOI improvements (full dataset)

*FOI: Follicle oocyte index, M2: Metaphase II*

Tailoring the FSH starting dose is typically guided by patient-specific characteristics and established ORTs. Individualized OS protocols have been supported by recent developments such as nomograms that assist clinicians in calculating daily FSH starting doses[8]. For women with normal ORTs, initial doses of 150-225 IU/day are commonly used, with the first ultrasound scan usually performed on the 5$^{th}$ or 6$^{th}$ day of stimulation. Subsequent dose adjustments, based on ovarian response, aim to prevent hypo-response or hyper-response. A recent systematic review reported that approximately 45% of cycles involved r-hFSH dose adjustments, with increases being more common than decreases[9]. However, it remains unclear whether initial doses or mid-cycle dose adjustments have a greater impact on follicular growth. Pharmacokinetic studies indicate that FSH reaches steady-state concentrations after 4–5 days of administration, with a biological response lag of approximately 4 days. This suggests that correct starting doses and fixed-dose regimens may better align with follicular dynamics than frequent dose adjustments, a hypothesis supported by recent data[10,11]. Further randomized controlled trials are needed to validate these findings[12].

**Table 2.** Ovarian stimulation outcomes of standard and tailored groups

| Parameter | Stat type | Standard group | Tailored group | p-value |
|---|---|---|---|---|
| Peak serum estradiol (pg/mL) | Median (minimum, maximum) | 1885.00 (273.00, 4228.00) | 2866.50 (1000.00, 8534.00) | 0.0000* |
| Gonadotropin starting dose (IU) | Median (minimum, maximum) | 150.00 (150.00, 225.00) | 300.00 (225.00, 300.00) | 0.0000* |
| Total consumed gonadotropins (IU) | Median (minimum, maximum) | 2250.00 (1050.00, 18750.00) | 2925.00 (1800.00, 5100.00) | 0.0000* |
| Total stimulation duration (day) | Median (minimum, maximum) | 11.00 (6.00, 17.00) | 11.00 (8.00, 17.00) | 0.258 |
| No of follicles >17 mm on trigger day | Median (minimum, maximum) | 5.00 (1.00, 13.00) | 9.00 (1.00, 22.00) | 0.0000 |
| FORT (%) | Median (minimum, maximum) | 0.44 (0.08, 1.50) | 0.69 (0.09, 1.50) | 0.0000** |
| FOI (%) | Median (minimum, maximum) | 0.38 (0.05, 0.81) | 0.67 (0.09, 1.00) | 0.0000* |
| No of COC | Median (minimum, maximum) | 5.00 (1.00, 9.00) | 9.00 (1.00, 20.00) | 0.0000* |
| No of M2 oocytes | Median (minimum, maximum) | 4.00 (1.00, 9.00) | 7.00 (1.00, 21.00) | 0.0000* |
| No of available day 3 embryos | Median (minimum, maximum) | 2.00 (1.00, 9.00) | 5.00 (1.00, 17.00) | 0.0000* |
| No of available day 5 embryos | Median (minimum, maximum) | 2.00 (0.00, 7.00) | 3.00 (0.00, 10.00) | 0.0000* |
| No of available top quality blastocysts | Median (minimum, maximum) | 0.00 (0.00, 3.00) | 2.00 (0.00, 5.00) | 0.0000* |

FORT: Follicular output ratio, FOI: Follicle oocyte index, COC: Cumulus oocyte complex, IU: International unit, M2: Metaphase II

**Table 3.** Comparison results for dose impact on M2 and FOI (%)

| Metric | Group | Mean (95% CI) | p-value | Significant | Absolute difference (95% CI) |
|---|---|---|---|---|---|
| FOI (%) | Standard *vs.* tailored | 0.34 (0.14, 0.54) | 0.0000 | Yes | 0.34 (0.14, 0.54) |
| M2 count | Standard *vs.* tailored | 3.88 (3.69, 4.08) | 0.0000 | Yes | 3.88 (3.69, 4.08) |

FOI: Follicle oocyte index, CI: Confidence interval, M2: Metaphase II

Higher gonadotropin doses improve oocyte yields, as shown in randomized control trials (RCTs) where dose increases of 50-100 IU resulted in significantly more oocytes, in unexpected responders and normal responders under 39 years[13-16]. While increasing gonadotropin doses may enhance ovarian response, studies indicate a potential increase in maternal complications, such as gestational diabetes mellitus and hypothyroidism, particularly in singleton pregnancies. This finding underscores the importance of individualized stimulation protocols[17]. Recent studies have shown that increasing recFSH doses by 50 IU increments improves oocyte retrieval outcomes in unexpected hypo-responders[12]. Our study confirmed these findings, with tailored dosing increases of 75-150 IU per patient and a maximum dose of 300 IU per day, resulting in significant improvements in oocyte yield and embryo outcomes[14,16]. This decision was based on our center's policy of not exceeding the generally accepted maximum dose of 300 IU/day to avoid diminishing returns and higher costs. However, recent evidence suggests that higher gonadotropin doses do not necessarily lead to an increased retrieval of MII oocytes. A machine learning model analyzing 9,598 ovarian stimulations demonstrated that excessive gonadotropin dosing may reduce oocyte yield in certain patient groups, reinforcing the importance of precise dosage optimization in clinical practice[18]. These findings highlight the benefits of tailored 'starting' dosing in unexpected hypo responders. While dose escalation may slightly increase costs, the higher number of embryos obtained enhances cumulative live birth chances, potentially making it a more cost-effective strategy. Differences in dose increments may reflect variations in patient BMI, which influences gonadotropin requirements. Until a consensus on optimal dose adjustments is established, decisions on escalation will depend on individual patient characteristics and clinical judgment.

The etiology of unexpected hypo response remains multifactorial, with emerging evidence pointing to genetic polymorphisms in gonadotropin receptors, such as the FSHR Ser680 allele and luteinizing hormone (LH)β variant, as significant contributors[19,20]. These polymorphisms are associated with altered gonadotropin receptor sensitivity, which may necessitate tailored stimulation approaches. In such cases, increasing gonadotropin doses or adding LH activity during OS has been suggested to improve follicular response and oocyte yield[20,21]. In a recent meta-analysis, significantly higher clinical pregnancy rates, implantation rates, and number of oocytes retrieved were observed in hypo-responders supplemented with recombinant LH versus hypo-responders who underwent FSH monotherapy[21]. However, no significant difference was observed between patients regarding the number of M2 oocytes retrieved. Therefore, there is a need for large controlled RCTs to provide robust evidence in this area.

Finally, variability in AFC measurements due to sonographer skill and ultrasound equipment differences poses a challenge in accurately identifying hypo-responders[8]. Nonetheless, the homogeneity of the study cohort minimizes some of these confounding factors, ensuring more reliable comparisons.

### Study Limitations

The retrospective design of this study and the use of historical controls may introduce bias related to inter-individual variations. Additionally, dose adjustments were based on BMI and institutional protocols, which may limit the generalizability of the findings. Furthermore, the absence of an LH supplementation arm represents an additional limitation of this study.

## Conclusion

Individualized gonadotropin dosing represents a promising approach for managing unexpected hypo responders, leading to enhanced ovarian response and improved embryological outcomes. Further randomized controlled trials are necessary to establish standardized protocols and optimize treatment strategies for this challenging patient subgroup.

### Ethics

**Ethics Committee Approval:** The study was approved by the Üsküdar University Faculty of Medicine Ethics Committee on October 26, 2020 (protocol number: 61351342/OCTOBER 2022-24).

**Informed Consent:** All participants provided informed consent before entering the study.

### Footnotes

### Authorship Contributions

Surgical and Medical Practices: E.P, R.P., Concept: B.P.K., E.P, Design: E.P., B.P.K, Data Collection or Processing: G.İ, D.H.D., E.P, Analysis or Interpretation: B.P.K, E.P, Literature Search: B.P.K., G.İ., Writing: B.P.K., D.H.D.

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

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# Effect of deep infiltrative endometriosis surgery and surgical method on sexual function in females

## *Kadınlarda derin infiltratif endometriozis cerrahisi ve cerrahi yönteminin cinsel fonksiyon üzerine etkisi*

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### Abstract

**Objective:** Sexual function and quality of life are significantly reduced in endometriosis patients, particularly those with deep infiltrative endometriosis (DIE). The purpose of this study was to compare the effects of endometriosis excision and excision techniques on sexual function among individuals with DIE to those of healthy females in an objective manner.

**Materials and Methods:** Our study included 140 individuals who were diagnosed as having DIE and reported dyspareunia in our clinic between January 2018 and 2024, and 70 patients who presented to our family planning clinic. The preoperative and 6-month post-surgery scores of the female sexual function index (FSFI), quality of sexual experience scale (QSES), and visual analog scale (VAS) values of all patients who described preoperative dyspareunia were examined retrospectively from the patient files.

**Results:** In our study, the FSFI score of healthy groups was seen to be significantly greater than the pre-surgery and post-surgery groups ($p<0.001$ and $p<0.001$, respectively). The QSES scores of the healthy group were found to be significantly higher than the pre-surgery and post-surgery groups ($p<0.001$ and $p<0.001$, respectively). The VAS dyspareunia values of the healthy group were discovered to be significantly lower than the pre-surgery and post-surgery groups ($p<0.001$ and $p<0.001$, respectively). The FSFI and QSES scores of the post-surgery group were significantly higher than those of the pre-surgery group ($p<0.001$ and $p<0.001$, respectively). The VAS dyspareunia score of the post-surgery group was seen to be significantly lower than that of the pre-surgery group ($p<0.001$). The FSFI and QSES scores of patients who underwent laparotomy were discovered to be significantly greater than that of individuals who underwent laparoscopic surgery ($p<0.001$ and $p=0.01$, respectively).

**Conclusion:** The surgical approach may have a positive effect on both organ dysfunction and sexual function in women with DIE; this issue should be considered carefully.

**Keywords:** Deep infiltrative endometriosis, endometriosis, sexual function

**PRECIS:** The surgical approach may have a positive impact on both organ dysfunction and sexual function in women with deep infiltrative endometriosis, and we therefore support the inclusion of women's sexual health issues in standard gynecological care.

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**Öz**

**Amaç:** Endometriozis varlığında, özellikle de derin infiltratif endometriozis (DIE) varlığında cinsel işlev ve yaşam kalitesi önemli ölçüde azalır. Bu çalışmanın amacı, endometriozis eksizyonu ve eksizyon tekniklerinin DIE'li bireylerde cinsel fonksiyon üzerindeki etkilerini sağlıklı kadınlarla objektif karşılaştırmaktır.

**Gereç ve Yöntemler:** Çalışmamıza Ocak 2018-2024 tarihleri arasında kliniğimizde DIE tanısı almış ve disparoni bildiren 140 kişi ve aile planlaması kliniğimize başvuran 70 hasta dahil edildi. Ameliyat öncesi disparoni tanımlayan tüm hastaların ameliyat öncesi ve ameliyat sonrası 6. ayda female sexual function indeks (FSFI), cinsel deneyim kalitesi ölçeği (QSES) ve görsel analog ölçek (VAS) değerleri hasta dosyalarından retrospektif olarak incelendi.

**Bulgular:** Çalışmamızda sağlıklı grubun FSFI skorunun ameliyat öncesi ve sonrası gruplardan anlamlı olarak yüksek olduğu saptandı (p<0,001 ve p<0,001, sırasıyla). Sağlıklı grubun QSES skorlarının ameliyat öncesi ve sonrası gruplardan anlamlı olarak yüksek olduğu saptandı (p<0,001 ve p<0,001, sırasıyla). Sağlıklı grubun VAS disparoni değerlerinin ameliyat öncesi ve sonrası gruplardan anlamlı olarak düşük olduğu saptandı (p<0,001 ve p<0,001, sırasıyla). Ameliyat sonrası grubun FSFI ve QSES skorlarının ameliyat öncesi gruptan anlamlı olarak yüksek olduğu saptandı (p<0,001 ve p<0,001, sırasıyla). Ameliyat sonrası grubun VAS disparoni skorunun ameliyat öncesi gruba göre anlamlı olarak düşük olduğu saptandı (p<0,001). Laparotomi geçiren hastaların FSFI ve QSES skorlarının laparoskopik cerrahi geçiren bireylere göre anlamlı olarak yüksek olduğu saptandı (p<0,001 ve p=0,01, sırasıyla).

**Sonuç:** Cerrahi yaklaşımın DIE'li kadınlarda hem organ disfonksiyonu hem de cinsel fonksiyon üzerinde olumlu bir etkisi olabilir ve bu nedenle bu konu dikkatlice ele alınmalıdır.

**Anahtar Kelimeler:** Derin infiltratif endometriosis, endometriozis, cinsel fonksiyon

## Introduction

The gynecologic condition endometriosis is characterized by the persistent inflammation and existence of tissue outside the uterus that resembles endometrial tissue[1]. About 10% of women who are of reproductive age and exhibit pelvic discomfort and infertility are thought to have endometriosis[2,3]. A kind of endometriosis that extends more than 5 mm below the peritoneal surface is known as deep infiltrative endometriosis (DIE)[4]. It is well acknowledged that endometriosis and specifically DIE, significantly lowers quality of life and sexual function[5-7]. Changes in sexual function in females with DIE may be caused by a variety of causes, including as tissue fibrosis, discomfort, chronic inflammation, and the presence of neuroactive drugs[8,9]. Since endometriosis is a benign condition, pain management and symptom improvement should be the primary objectives of treatment. Conservative approaches, like medical care, may be risk-free and innocuous, but they frequently don't work, particularly in DIE[10,11].

As long as endometriotic foci are completely eliminated, symptomatic endometriosis surgery is usually successful and has a low recurrence rate[12,13]. On the other hand, intestinal or urinary neurogenic dysfunction might make DIE laparoscopic treatment more difficult, particularly in cases where nerve-sparing surgery is not an option[14,15]. We aimed in this retrospective study to objectively evaluate the effect of endometriosis excision and excision methods on sexual function in individuals having DIE in comparison to healthy females.

## Materials and Methods

The present study was conducted as a retrospective observational study pursuant to the guidelines of the Helsinki Declaration. Documents of informed consent were taken from all patients. The research was approved by the Ethics Committee of İzmir Democracy University Hospital (date: 26/06/2024, number: 2024/20-5). Our study included 140 patients who were diagnosed as having DIE and reported dyspareunia in our clinic between January 2018 and 2022, and 70 patients who presented to our clinic for birth control. Before surgery, all patients with DIE underwent transvaginal and abdominal ultrasonography as well as gynecologic tests to determine whether they had pelvic endometriosis[16,17]. After retrospective screening of patients who underwent surgery due to DIE, 70 patients who underwent laparoscopic surgery (L/S) and 70 patients who underwent laparotomy (L/T) were included in the study.

The preoperative female sexual function index (FSFI)[18], quality of sexual experience scale (QSES)[19], and visual analog scale (VAS)[20] values of all patients who described preoperative dyspareunia were examined retrospectively from the patient files. Females with DIE had complete excision of macroscopic endometriotic lesions in accordance with relevant surgical procedures[21,22]. The same surgeon carried out the surgeries while the surgical team had consistently treated DIE patients with laparoscopic and laparotomic procedures in the past. Histologic analysis was performed on all patients after surgery, and only patients whose diagnosis was confirmed were included in the research. After 6 months, all participants had clinical examinations and transvaginal ultrasonography to evaluate the symptoms and/or anatomic recurrence of endometriotic nodules. Six months after surgery, the FSFI, QSES, and VAS scores of all patients were retrospectively examined from their patient files. On the VAS scale, 0 represents the lack of symptoms and 10 represents the worst conceivable illness. Dyspareunia was scored by participants on a range of 0 to 10.

The FSFI assessed six distinct domains: pain/discomfort, satisfaction, orgasm, lubrication, arousal, and desire. The scale ranged from 0, indicating (no sexual activity in the previous four weeks or a score of) 1 (very unhappy) to 5 (very satisfied). A full-scale score ranging from 2.0 (severe dysfunction) to 36.0 (no dysfunction) was used in the study to assess sexual function, with higher FSFI scores thought to be linked to improved symptoms. According to Wiegel et al.[23], there is an

ideal cut-off score of 26, which is utilized to identify females who have sexual dysfunction from those who do not.

Greater QSES scores correspond to greater quality. The range of values is 7 to 49[24]. This questionnaire also examines several facets of sexual life and the influence of pelvic issues on sexual functioning. When responding to the questions, participants were instructed to take into account the preceding four weeks. Exclusion criteria for the study were a history of gynecologic cancer, presence of depression, patients receiving postoperative medical treatment, inflammatory bowel disease, a record of pelvic radiotherapy or systemic chemotherapy, and gynecologic infection based on demographic and clinical characteristics [parity, body mass index (BMI), age].

### Statistical Analysis

Statistical analysis was completed with the SPSS 26.0 software package (IBM Inc., Chicago, IL, USA). The distribution normality was evaluated using the Kolmogorov-Smirnov test. Non-normally-normally distributed parameters were analyzed with the Mann-Whitney U test. The Wilcoxon test was employed to determine changes before and after surgery. For the analysis of categorical data, the chi-square test and Fisher precision test were utilized. Regarding the statistical analysis, categorical variables are reported as percentages, and quantitative variables as median (minimum-maximum). Statistical significance was considered as $p<0.05$ and analyses were within 95% confidence intervals.

## Results

The average age of the participants in our study was 30.1±9.5 years and the BMI average was 24.1±5.9 kg/m$^2$. Fifty-one (24.3%) of the participants in our study had given birth. In this research, the average BMI of patients who underwent endometriosis surgery was 23.9±5.6 kg/m$^2$, which was significantly lower than that of healthy females ($p<0.001$) (Table 1).

The FSFI score of the healthy group was significantly greater than the pre-surgery and post-surgery groups ($p<0.001$ and $p<0.001$, respectively). The QSES score of the healthy group was significantly greater than the pre-surgery and post-surgery groups ($p<0.001$ and $p<0.001$, respectively). The VAS dyspareunia score of the healthy group was seen to be significantly lower than the pre-surgery and post-surgery groups ($p<0.001$ and $p<0.001$, respectively). The FSFI score of the post-surgery group was significantly greater than the pre-surgery group ($p<0.001$). The QSES score of the post-surgery group was significantly higher than the pre-surgery group ($p<0.001$). The VAS dyspareunia score of the post-surgery group was significantly lower than that of the pre-surgery group ($p<0.001$) (Table 2).

**Table 1.** Comparison of demographic characteristics of main groups

| Variables | All patients (n=210) (100%) | Healthy females (n=70) (33.3%) | Surgery (+) (n=140) (66.7%) | p* |
|---|---|---|---|---|
| | Mean ± SD | | | |
| **Age (years)** | 30.1±9.5 | 30.2±9.4 | 30.1±9.6 | 0.5 |
| **BMI (kg/m²)** | 24.1±5.9 | 24.5±5.4 | 23.9±5.6 | <0.001 |
| **Birth history** | 51 (24.3%) | 22 (31.4%) | 29 (20.7%) | 0.08 |

*p value: Mann-Whitney U test, BMI: Body mass index, SD: Standard deviation

**Table 2.** Comparisoniof scores of healthy females and females with a history of surgery

| Variables | Healthy females (n=70) (33.3%) | Pre-surgery (n=140) (66.7%) | Post-surgery (n=140) (66.7%) | p* | p** | p*** |
|---|---|---|---|---|---|---|
| **FSFI** | 29 (23-36) | 26 (21-32) | 28 (25-34) | <0.001 | <0.001 | <0.001 |
| **QSES** | 43 (36-46) | 40 (32-45) | 41 (33-46) | <0.001 | <0.001 | <0.001 |
| **VAS (dyspareunia)** | 2 (0-7) | 4 (2-10) | 3 (1-5) | <0.001 | <0.001 | <0.001 |
| **Desire** | 5 (4-6) | 4 (4-5) | 4 (4-6) | <0.001 | <0.001 | 0.08 |
| **Arousal** | 5 (4-6) | 4 (3-5) | 5 (4-6) | <0.001 | 0.2 | <0.001 |
| **Lubrication** | 5 (4-6) | 4 (3-5) | 4 (3-6) | <0.001 | 0.03 | <0.001 |
| **Orgasm** | 5 (3-6) | 4 (3-6) | 5 (4-6) | <0.001 | 0.3 | <0.001 |
| **Satisfaction** | 5 (4-6) | 4 (3-6) | 5 (4-6) | <0.001 | 0.06 | <0.001 |
| **Pain** | 5 (4-6) | 4 (3-6) | 5 (4-6) | <0.001 | 0.1 | <0.001 |

p*: Healthy *vs.* pre-surgery (Mann-Whitney U test), p**: Healthy *vs.* post-surgery (Mann-Whitney U test), p***: Pre-surgery *vs.* post-surgery (Wilcoxon test), FSFI: Female sexual function index, QSES: Quality of sexual experience scale, VAS: Visual analog scale

In the present research, the FSFI scores of individuals who had L/T were significantly higher than those of patients who underwent L/S (p<0.001). The QSES scores of patients who underwent L/T were significantly higher than those of patients who underwent L/S (p<0.001). Arousal scores of individuals who had L/T were significantly higher than those of patients who underwent L/S (p=0.009). The orgasm scores of patients who underwent L/T were significantly higher than those of patients who underwent L/S (p=0.01). Satisfaction scores of patients who underwent L/T were significantly higher than those of patients who underwent L/S (p<0.001). Pain scores of individuals who had L/T were significantly higher than those of patients who underwent L/S (p=0.01) (Table 3).

**Table 3.** Comparisoniof score changes in individuals who underwent laparoscopy and laparotomy

| Variables | Laparoscopy (n=70) (50%) | Laparotomy (n=70) (50%) | p* |
|---|---|---|---|
| **Pre-FSFI** | 25 (21-31) | 26 (21-31) | 0.4 <0.001 |
| **Post-FSFI** | 28 (25-32) | 29 (25-34) | |
| **p**** | <0.001 | <0.001 | |
| **Pre-QSES** | 40 (32-45) | 40 (33-45) | 0.3 0.01 |
| **Post-QSES** | 41 (33-45) | 41 (34-46) | |
| **p**** | <0.001 | <0.001 | |
| **Pre-VAS (dyspareunia)** | 4 (2-10) | 4.5 (2-10) | 0.8 0.2 |
| **Post-VAS (dyspareunia)** | 3 (2-5) | 3 (1-5) | |
| **p**** | <0.001 | <0.001 | |
| **Pre-desire** | 4 (4-5) | 4 (4-5) | 0.6 0.4 |
| **Post-desire** | 4 (4-6) | 5 (4-6) | |
| **p**** | 0.2 | 0.1 | |
| Pre-arousal | 4 (3-5) | 4 (3-5) | 0.2 0.009 |
| Post-arousal | 5 (4-5) | 5 (4-6) | |
| p** | 0.02 | <0.01 | |
| **Pre-lubrication** | 4 (3-5) | 4 (3-5) | 0.07 0.2 |
| **Post-lubrication** | 5 (4-6) | 5 (4-6) | |
| **p**** | <0.001 | <0.001 | |
| **Pre-orgasm** | 4 (3-5) | 4 (3-6) | 0.7 0.01 |
| **Post-orgasm** | 5 (4-5) | 5 (4-6) | |
| **p**** | <0.001 | <0.001 | |
| **Pre-satisfaction** | 4 (4-6) | 4 (3-6) | 0.5 <0.001 |
| **Post-satisfaction** | 4 (3-6) | 5 (4-6) | |
| **p**** | <0.001 | <0.001 | |
| **Pre-pain** | 4 (3-5) | 4 (3-6) | 0.02 0.01 |
| **Post-pain** | 5 (4-5) | 5 (4-6) | |
| **p**** | <0.001 | <0.001 | |

p*: Mann-Whitney U test, p**: Wilcoxon test, FSFI: Female sexual function index, QSES: Quality of sexual experience scale, VAS: Visual analog scale

## Discussion

In this current research, we hypothesized that surgical treatment of females with DIE may have a positive effect on FSFI and QSES scores and dyspareunia, indicating improvement in overall sexual function. Our goal was to compare the sexual functioning of female individuals with DIE to that of healthy ones. Numerous local environmental, psychological, neurologic, and biological elements that impact one's bodily and mental well-being, as well as one's perception of femininity and interpersonal interactions, are all involved in sexual function[25,26]. In addition, depression and infertility, that are very common in females having endometriosis, are also linked with sexual dysfunction[27,28]. Recently, there has been increased medical interest in the impact of the presence of endometriosis on sexual function in females. Numerous research in the literature has shown that patients having dyspareunia experience a marked reduction in severity and an improvement in their quality of sexual function following surgical excision of DIE lesions[29,30]. Combining L/S with postoperative hormonal treatment is beneficial for females with endometriosis, especially DIE, as it has been demonstrated to enhance sexual function and symptoms[31,32]. In line with these studies, our results revealed a notable enhancement in sexual function six months following treatment in patients treated for DIE. There are numerous questionnaire formats for examining sexual activity in females with endometriosis[33-35]. In our study, we used the FSFI survey. We preferred it because of its succinctness, proven validity, and trustworthy subscales (sexual desire, pain/discomfort, sexual arousal, satisfaction, orgasm, lubrication).

Furthermore, we evaluated QSES survey data. In our study, in addition to a significant increase in FSFI, QSES, and VAS scores after surgery, found a significant improvement in FSFI subcategories. However, despite having no significant difference in FSFI subcategory values between patients in the post-surgery group and the healthy group, FSFI, QSES, and VAS values in the healthy group were still greater than the post-surgery group scores.

Sexual desire and satisfaction are deeply influenced by emotions and governed by a range of excitatory and inhibitory impacts. The most important of these obstacles is the presence of dyspareunia. For this reason, the VAS dyspareunia score among patients in our study were also evaluated using a questionnaire. Because pain is both a potent inhibitor of the sexual response cycle and a potent behavioral modulator, females with dyspareunia are more likely to develop hypoactive sexual desire disorder or arousal problems[36]. Presumably because they were aware that endometriotic lesions had been eliminated and their post-operative pain sensations had decreased and they were more aware that endometriotic lesions had been eliminated, the women in the post-surgery group were able to unwind and feel

more at ease during sexual activity. It has been demonstrated that the number of DIE nodules detected in certain regions is inversely correlated with the decrease in sexual desire[37].

At advanced stages, the illness can impact a variety of areas of a woman's life, including her mental health. According to reports, endometriosis-afflicted women have greater rates of psychological disorders such as anxiety and depression[38]. Furthermore, there is a substantial correlation between DIE and persistent pelvic discomfort. Taking into account various studies, there is a substantial correlation between the cumulative size of posterior DIE (less than 1 cm) and both chronic pelvic discomfort and the least severe dyspareunia[39]. The involvement of the anterior rectal wall, posterior vaginal fornix, pouch of Douglas, and uterosacral ligaments was observed in two separate investigations by Kor et al.[39] and Vercellini et al.[40]. The degree of Douglas pouch stenosis and the endometriosis stage are associated with the severity of dyspareunia. Our study's results, which corroborate the findings of the other two studies, show a considerable improvement in VAS dyspareunia ratings following surgery. This finding might be explained by the fact that sexual dysfunction related to pelvic issues can be lessened by fully removing all endometriotic lesions and restoring normal pelvic structure. However, we assessed each of the FSFI score subgroups independently and did not restrict the assessment of sexual function to the existence or absence of pelvic discomfort. In particular, we found that the desire scale did not significantly improve following surgical therapy for DIE. Since sexual desire is a multifaceted process that depends on psychological, anatomical, and physiological components, it is challenging to explain this conclusion[41]. With respect to anatomical variables, it is well recognized that undergoing extensive surgery for endometriosis may result in injury to the autonomic nerves, which might subsequently affect orgasm[42]. In this current research, the higher orgasm score in females with a history of L/T in the post-surgery group than in the L/S group suggests that nerve damage is more limited in the L/T group.

### Study Limitations

We are aware that it is important to take into account the limitations of this research when evaluating the findings. The research was designed retrospectively; patient data were analyzed only from patient files and data in the database; and the limited number of patients can be cited as limitations of the study. Also, the majority of patients at our tertiary care center for endometriosis therapy were in a severe stage of illness; as a result, research participants may not be entirely typical of the endometriosis community as a whole. Patients with DIE are more prone than those with less severe illness to experience sexual dysfunction. Because the FSFI and QSES questionnaires inquire about areas of sexual function that many women view as extremely private, there is a considerable risk of response and recall bias, even though women complete them independently and without guidance. The psychological elements that characterize human sexuality and the larger cultural background make studies of human sexuality vulnerable to prejudice and confounding variables. Nevertheless, our study has several strengths. Unlike other studies in the literature, our research assessed only the impact of DIE surgery on sexual function and individuals who had medical treatment in the postoperative period were not included in the study. In this way, the study made it possible to assess the impact of DIE surgery alone on sexual function, independent of medical treatment. Furthermore, unlike other studies in the literature, the type of surgical method was also evaluated as a factor in our study. Finally, all surgical patients were diagnosed with histologically confirmed DIE.

## Conclusion

We found that females affected by DIE generally had significant improvement in their postoperative sexual function compared with the preoperative period. FSFI subparameters and QSES scores improved significantly following surgical treatment. For females affected by DIE, the surgical treatment may improve both organ failure and sexual function; therefore, this issue should be considered carefully.

### Ethics

**Ethics Committee Approval:** The research was approved by the Ethics Committee of İzmir Democracy University Hospital (date: 26/06/2024, number: 2024/20-5).

**Informed Consent:** Documents of informed consent were taken from all patients.

### Footnotes

### Authorship Contributions

Surgical and Medical Practices: U.A., A.C.Ö., Concept: O.Y., C.A., F.A., Design: H.A.A., T.B.B., Data Collection or Processing: S.E., A.C.Ö., Analysis or Interpretation: H.A.A., T.B.B., Literature Search: F.A., Writing: U.A., B.E.

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# Assessing the potential of nifedipine and resveratrol to enhance ovarian viability in conjunction with detorsion treatment: A rat ovarian torsion model study

## *Detorsiyon tedavisine ek olarak nifedipin ve resveratrolün serum anti-müllerian hormon seviyesi ve over histopatolojisi üzerindeki etkileri: Rat over torsiyon modeli çalışması*

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### Abstract

**Objective:** Evaluating the therapeutic effect of detorsion, resveratrol, and nifedipine on ovarian viability assessed by biochemical, histopathological and immunohistochemical parameters and markers of oxidative stress.

**Materials and Methods:** Twenty-four Sprague-Dawley rats were included in 4 groups, namely: sham operation, ischemia-reperfusion (I/R), I/R+10 mg/kg nifedipine (NIF), I/R+100 mg/kg resveratrol (RSV). In the study groups, bilateral 720º ovarian torsion was performed and continued for 3 hours, followed by detorsion for another 3 hours. Thirty minutes before the detorsion, NIF and RSV groups received respective treatments. Adnexectomy was performed, and evaluations were made for the expression of anti-müllerian hormone (AMH), vascular endothelial growth factor receptor 2 (VEGFR-2), markers of oxidative stress, and follicle counts. Blood AMH levels were measured.

**Results:** No change in AMH levels was detected. Although the expression of AMH was significantly reduced following I/R alone, it remained similar to the control group in the NIF group. Meanwhile, the RSV group exhibited slightly lower expression than the control, although it was still higher than that observed with the I/R injury group. VEGFR-2 staining was similar in the I/R and NIF groups, but reduced in the RSV group. Markers of oxidative stress were similar between groups. Primordial follicle count was lower in the untreated I/R injury group compared to the control group ($p<0.05$). The NIF group had more secondary follicles than the I/R injury and RSV groups ($p<0.05$).

**Conclusion:** Nifedipine and resveratrol treatments did not influence AMH levels and antioxidant-oxidant system parameters in rats exposed to I/R injury. However, nifedipine had a positive effect on AMH expression and secondary follicle count, and resveratrol decreased the expression of VEGFR-2 in tissues, which can be an indicator of their clinical potential.

**Keywords:** Ovary, follicle count, anti-müllerian hormone, VEGFR-2, immunohistochemical analysis, oxidative stress

**PRECIS:** We evaluated the protective effect of nifedipine and resveratrol in an experimental rat adnexal I/R injury model by creating subsequent adnexal torsion and detorsion.

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**Öz**

**Amaç:** Bu çalışmanın amacı, detorsiyon, resveratrol ve nifedipin tedavisinin anti-müllerian hormon (AMH) seviyeleri ve over histopatolojisi üzerindeki etkisinin değerlendirilmesidir.

**Gereç ve Yöntemler:** Yirmi dört Sprague-Dawley rat; kontrol, iskemi-reperfüzyon (I/R), I/R+10 mg/kg nifedipin (NIF), I/R+100 mg/kg resveratrol (RSV) gruplarına ayrıldı. Çalışma gruplarında, bilateral 720º over torsiyonu yapıldı ve 3 saat boyunca tutuldu, ardından 3 saat boyunca detorsiyon uygulandı. Detorsiyondan otuz dakika önce, NIF ve RSV gruplarına ilgili tedaviler uygulandı. Adneksiyal ekizyon yapıldı ve AMH ve vasküler endotelyal büyüme faktörü reseptörü 2 (VEGFR-2) immünohistokimyasal ekspresyonu, oksidatif stres belirteçleri ve folikül sayıları değerlendirildi. Kan AMH seviyeleri ölçüldü.

**Bulgular:** AMH seviyelerinde değişiklik tespit edilmedi. AMH ekspresyonu I/R grubunda azalmış olmasına rağmen, NIF grubunda kontrol ile benzerdi. RSV grubunda ekspresyon, I/R grubuna göre daha yüksek, ancak kontrol grubuna göre daha düşüktü. VEGFR-2 boyaması, I/R ve NIF gruplarında kontrol grubuna benzerdi, ancak RSV grubunda azalmıştı. Gruplar arasında oksidatif stres belirteçlerinde istatistiksel olarak anlamlı bir fark tespit edilmedi. Kontrol grubundaki primordial folikül sayısı, I/R grubundan daha yüksekti ($p<0,05$). NIF grubundaki sekonder folikül sayısı, I/R ve RSV gruplarından daha yüksekti ($p<0,05$).

**Sonuç:** Nifedipin ve resveratrol tedavileri, ratlarda I/R hasarında AMH seviyeleri ve antioksidan-oksidan sistem parametrelerini etkilemedi. Ancak, nifedipin AMH ekspresyonu ve sekonder folikül sayısı üzerinde olumlu bir etkiye sahipti ve resveratrol, dokularda VEGFR-2 ekspresyonunu azalttı, bu da bu ajanların klinik potansiyellerinin bir göstergesi olabilir.

**Anahtar Kelimeler:** Yumurtalık, folikül sayısı, anti-müllerian hormon, VEGFR-2, immünohistokimyasal analiz, oksidatif stres

## Introduction

Adnexal torsion is a serious gynecological emergency defined by the ovary rotating partially or fully around its pedicle or vascular axis. Torsion creates lymphatic and arterial blockage that may result in gangrene and necrosis in adnexa[1]. Ovarian cysts represent the predominant cause of ovarian torsion[2]. A sudden occurrence of abdominal pain, often with nausea or vomiting, frequently indicates ovarian torsion[3]. In case of a suspected ovarian torsion, the initial imaging technique used is ultrasound. Impaired or absent blood flow in an enlarged and edematous ovary on ultrasound is findings that assist in the diagnosis of ovarian torsion[4]. Historically, adnexectomy was considered to be the only option. Nevertheless, results from contemporary studies suggest that detorsion of the adnexa can be a more conservative treatment option with similar success[1]. However, concerns have been raised about increased production of reactive oxygen radicals due to reperfusion after detorsion, which can inflict further damage to the adnexa[5]. Various agents have been suggested to reduce this ischemia-reperfusion (I/R) injury, including nifedipine and resveratrol[6-8].

Resveratrol, naturally a phytoalexin, is typically found in plants such as grapes and berries, and is thought to have an antioxidant effect due to the hydroxyl group it contains[9]. Meanwhile, nifedipine is a calcium channel blocker, and its effect on reducing oxygen radicals formed after I/R injury has been tested in several studies on the heart and testis[7,10-12]. Few studies have examined the antioxidant effect of resveratrol in protecting the adnexa from I/R injury following a detorsion, and none have investigated nifedipine, to the best of our knowledge[8].

Our objective was to examine the effect of nifedipine and resveratrol on ovarian viability in a rat model of adnexal I/R injury, induced through consecutive adnexal torsion and detorsion procedures. Ovarian viability was evaluated with biochemical, histopathological and immunohistochemical parameters and markers of oxidative stress.

## Materials and Methods

The protocol was reviewed and approved by the Gazi University Animal Experiments Local Ethics Committee of animal studies (approval number: G.Ü.ET-20.034, date: 09.07.2020). All experiments were conducted in accordance with the ARRIVE guidelines, the U.K. Animals (Scientific Procedures) Act of 1986 and its associated guidelines, EU Directive 2010/63/EU for animal experiments, and the National Research Council's Guide for the Care and Use of Laboratory Animals.

In the study, we included 24 female Sprague-Dawley rats weighing 255-290 grams and about 3 months old, divided into 4 groups with the same number of rats in each group using the RAND function in Microsoft Excel (v16.0, Microsoft Corporation, Redmond, U.S.). Rats were taken under standard laboratory conditions with a temperature of 22±2° C, 60% relative humidity, and 12 hour light and dark photoperiod before the experiment at a standard temperature of 22° C for at least 48 hours prior to randomization, being fed with tap water ad libitum and standard rat chow.

Allocation concealment was maintained until allocation. Animal care staff were unaware of allocation groups. The surgeon performing the adnexectomy was blind to which treatment each rat received. During the analysis of the outcome, investigators knew which rats were grouped to enable group comparisons; however, they were unaware of which specific treatment each group received.

Groups were determined as follows: Group I, control (sham operation); Group II, torsion-detorsion (I/R); Group III, torsion-detorsion + nifedipine (I/R + NIF); Group IV, torsion-detorsion + resveratrol (I/R + RSV).

### Surgical Procedure

After weighing, anesthesia was performed with xylazine (5 mg/kg) and ketamine (45 mg/kg) intramuscular injections. The operation field was sterilized, and all rats were placed in the operation area on their backs. Blood samples were taken from all rats preoperatively for anti-müllerian hormone (AMH)

measurement. The torsion-detorsion model was performed according to Parlakgumus et al.'s[13] study. We twisted ovaries two complete circles around their pedicle as described in the method. Laparotomy started with a 2-cm midline abdominal incision, and then intestines were gently retracted. The subsequent procedures were implemented for each group:

• Group I (sham operation): Bilateral adnexal exploration was performed without intervention following the midline laparotomy. Six hours after the laparotomy, a blood sample for AMH measurement was collected, and bilateral ovaries were surgically removed.

• Group II (I/R): After midline laparotomy, both adnexa were explored and then rotated clockwise by 720°. Then, both twisted adnexa were sutured to the lateral abdominal wall with a 5.0 polyglactin suture. The skin was closed with a 4.0 poliglecapron suture. Three hours after torsion, a secondary laparotomy was performed to achieve detorsion of the adnexa to provide reperfusion (Figure 1). The abdomen was closed after detorsion. Three hours following reperfusion, blood samples were taken for AMH measurement, and bilateral adnexa were surgically removed.

• Group III (I/R + NIF): Exact steps from Group II were followed, except, 30 minutes before detorsion, this group received intraperitoneal nifedipine (10 mg/kg). The dosage of nifedipine was based on Chander and Chopra[14] study, which assessed its protective effect against cyclosporine-induced oxidative stress in rats.

• Group IV (I/R + RSV): Exact steps from Group II were followed, except, 30 minutes before detorsion, this group received intraperitoneal resveratrol (100 mg/kg). The dosage of resveratrol was determined according to Aydın et al.'s[15] study, which evaluated its protective effect against sepsis-induced oxidative stress in rats.

### Biochemical AMH Evaluation

Serum samples were obtained by centrifuging blood samples at 4000 rpm for 10 minutes. Then all samples were preserved at -80 ºC in Eppendorf tubes in an ultra-low temperature freezer until analysis. AMH levels were analyzed on the same day with an automatic ELISA kit.

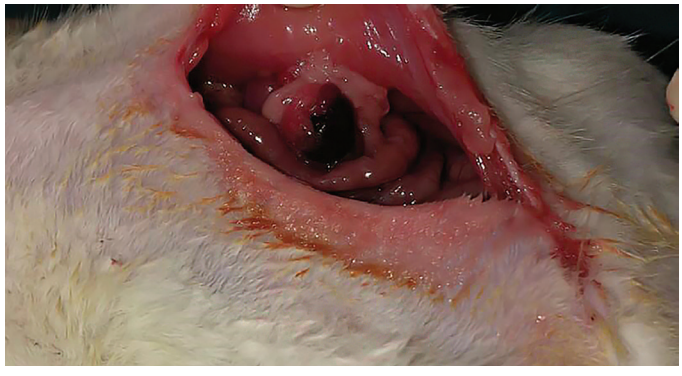

**Figure 1.** During secondary laparotomy three hours after torsion, ischemia is visible on the torsioned ovary

### Histopathological Examination

Ovaries were placed in fixation fluid containing 10% neutral buffered formalin. Afterwards, samples were embedded in paraffin and were processed using routine histological light microscopy techniques. Once embedded, sections were taken from each paraffin block to create 4-micron-thick slices. Ovary sections were stained with hematoxylin and eosin. Histomorphological analyses and follicle counting were performed using a light microscope and a computer-enhanced visualization system (Leica DM 4000, Germany). Primordial follicles, antral follicles, atretic follicles, and corpus luteum were counted. The number of follicles from five randomly selected cross-sections from both experimental and control groups was quantified, and the results were compared.

### Immunohistochemical Procedure

Anti-AMH antibody and anti-vascular endothelial growth factor receptor 2 (VEGFR-2) antibody were used for immunohistochemical evaluation based on the avidin-biotin peroxidase method. Ovarian tissue blocks were sectioned with a microtome into 4 micrometer-thick cross-sections. After deparaffinization, the cross sections underwent retrieval processing by incubation in citrate buffer (pH 6.0) (Lab Vision, Thermo Scientific) and exposure to 3% hydrogen peroxide (Lab Vision, Thermo Scientific) to block endogenous hydrogen peroxidase enzyme activity. Ultra V block (Lab Vision, Thermo Scientific, Fremont) was used to restrict non-selective interaction of enzymes or primary antibodies with tissue. Following the blocking stage, primary antibody (VEGFR-2 polyclonal antibody, bs10412R, Bios, AMH polyclonal antibody, D1201A, AFG Bioscience, respectively) was applied to tissue sections at a dilution ratio of 1:150 for a duration of 90 minutes. Following the primary antibody incubation, sections were washed with phosphate-buffered saline (PBS) three times for three minutes each. Afterwards, the secondary antibody (Lab Vision, Thermo Scientific, Fremont) was used for 10 minutes. The tissues were washed again with PBS three times for three minutes each. The immunohistochemical reaction components were made visible using streptavidin peroxidase complex (Lab Vision, Thermo Scientific, Fremont) with DAB. Counter-staining was performed with Mayer's hematoxylin. The immunostaining intensity in adnexal sections was evaluated semi-quantitatively as follows: (0) very weak; (1) weak; (2) moderate; and (3) strong[16,17].

### Evaluation of Oxidative Stress

Total antioxidant status (TAS) and total oxidant status (TOS) of ovarian tissues were measured using Erel's method[18]. The oxidative stress index (OSI) was calculated as OSI=0.25 TOS/TAS.

### Statistical Analysis

SPSS for Windows 22.0 (Statistical Package for the Social Sciences) software was utilized for statistical analysis. Normal distribution was evaluated by the Shapiro-Wilk test. Standard

deviations were used to present normally distributed data, while medians and percentiles were used for the rest. Categoric data were reported as percentages. Independent groups were compared with independent samples t-test for normally distributed data and with Mann-Whitney U test for non-parametric data. ANOVA was used to compare multiple groups, and post-hoc tests were conducted. The threshold for statistical significance was established at p<0.05.

## Results

### Biochemical AMH Evaluation

Preoperative to postoperative AMH level change was similar among all groups (p=0.058; p=0.089; p=0.756; p=0.206, respectively) (Table 1).

### Histopathological Examination

Number of follicles was similar, except that in primordial and secondary follicles (p=0.047, p=0.031, respectively) (Table 2). Post-hoc tests showed that the control group had a higher primordial follicle count than the I/R group (p=0.014). The nifedipine-treated group had a higher secondary follicle count compared to the I/R and I/R+RSV groups (p<0.013, p<0.017, respectively) (Table 3). Visualization of changes in follicle morphology and vascular dilatation is presented in Figure 2.

### Immunohistochemical Procedure

Immunohistochemical evaluation showed a remarkable decrease in AMH reactivity in the I/R group (Figure 3). Although AMH staining was more prominent in the resveratrol-treated group compared to the I/R group, it was observed to be weaker than in the control group. It was noted that in the nifedipine-treated group, tissue-wide uptake in the AMH immunostainings was similar to that in the control group.

Immunostaining for VEGFR-2 showed significantly increased immunoreactivity in all regions of the I/R group (Figure 4). VEGFR-2 levels were low in the resveratrol group, as in the control group. Conversely, levels in the nifedipine-treated group were close to those in the I/R group.

### Evaluation of TAS, TOS, and OSI

Assessment of TAS, TOS, and OSI in harvested ovarian tissues yielded no significant difference between groups (p=0.094, p=0.089, p=0.162, p=0.611) (Table 4).

## Discussion

We investigated the effects of nifedipine and resveratrol on enhancing ovarian viability following I/R injury due to torsion-detorsion. Histopathological findings indicated that the nifedipine-treated group exhibited a higher secondary follicle count compared to the I/R and I/R+RSV groups. Additionally, the AMH immunostaining grade in the nifedipine group was similar to the control group. Shown by VEGFR-2 staining, resveratrol also demonstrated protective attributes.

AMH level is an important parameter for predicting the reproductive period and evaluating long-term ovarian function in women; however, in many studies in the literature, only the postoperative AMH level was evaluated[19,20]. We hypothesized that evaluating the change of AMH level from the preoperative to the postoperative period can better portray the extent of I/R injury. Like our study, Karakaş et al.[21] investigated the effect of metformin on I/R injury in ovaries, focusing on blood AMH levels. The researchers found a higher AMH change in

**Table 1.** Preoperative to postoperative AMH change within each group

| | Group 1 (SD) | Group 2 (SD) | Group 3 (SD) | Group 4 (SD) |
|---|---|---|---|---|
| Preoperative AMH (ng/mL) | 1.94 (0.3) | 2.17 (0.4) | 2.23 (0.6) | 1.93 (0.2) |
| Postoperative AMH (ng/mL) | 1.64 (0.2) | 1.81 (0.4) | 2.32 (0.7) | 1.75 (0.5) |
| AMH change (ng/mL) | 0.3 (0.3) | 0.35 (0.4) | 0.9 (0.6) | 0.17 (0.3) |
| p-value | 0.058 | 0.089 | 0.756 | 0.206 |

SD: Standard deviation, AMH: Anti-müllerian hormone

**Table 2.** Evaluation of primordial, primary, secondary, antral corpus luteum and atretic follicle count

| | Group 1 (SD) | Group 2 (SD) | Group 3 (SD) | Group 4 (SD) | p-value |
|---|---|---|---|---|---|
| Primordial | 8.8 (2.6)[a] | 4.8 (1.9)[a] | 6.3 (2.5) | 6.3 (1.9) | 0.047 |
| Primary | 3.6 (0.8) | 2.8 (1.3) | 4.8 (0.9) | 4.8 (2.7) | 0.13 |
| Secondary | 3.6 (2.2) | 1.5 (0.7)[b] | 2.8 (0.7)[b, c] | 1.8 (0.4)[c] | 0.031 |
| Antral | 4.3 (0.8) | 2.6 (1.2) | 3.5 (1.3) | 3 (1.2) | 0.246 |
| Corpus luteum | 5.5 (2.07) | 5.1 (1.7) | 4.8 (1.07) | 5 (3.09) | 0.96 |
| Atretic | 17 (6.2) | 23 (11.1) | 16.8 (9.1) | 18.6 (6.5) | 0.541 |

SD: Standard deviation, ANOVA test. Superscript letters ([a, b, c]) indicate statistically significant difference between groups, detected in post-hoc analysis

the torsion/detorsion group, while the addition of metformin maintained the levels of the control. In the study of Sakin et al.[19], the effect of phosphodiesterase-5 inhibitors on ovarian I/R injury was investigated, and the postoperative AMH level was measured 6 hours after surgery, without measuring the preoperative AMH level. They reported that administration of vardenafil was associated with higher postoperative AMH levels; however, it could be argued that this result may be unreliable due to preoperative AMH levels being unknown.

Follicle count is an important parameter in most of the studies evaluating ovarian I/R injury. The increase in oxidative stress during ovarian torsion causes tissue damage, and reduction in follicle numbers in almost all stages of oocyte maturation[22]. Shokri et al.[23] analyzed the effect of Galea officinalis extract on

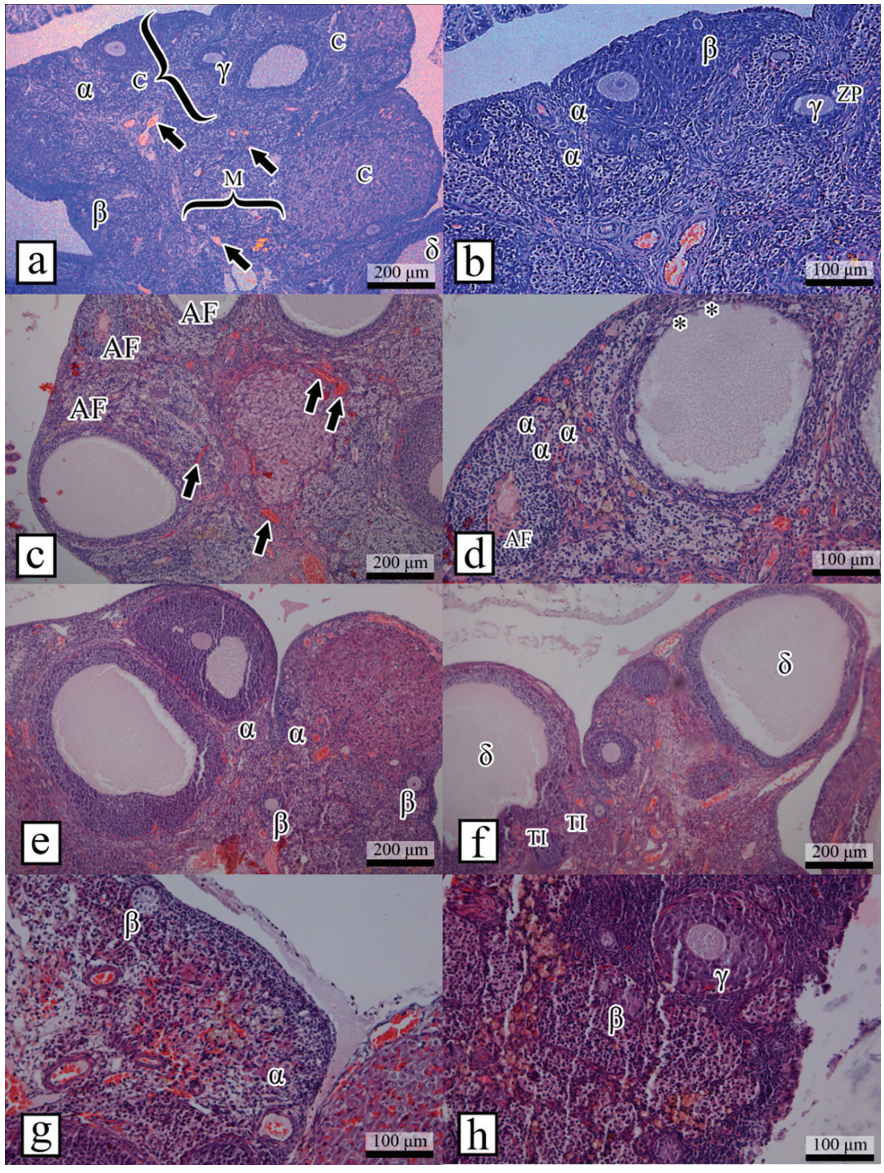


**Figure 2.** Representative micrographs of H&E-stained ovarian tissue sections captured under 100 x and 200 x magnifications, in all experimental groups: Group I (2a, 2b); Group II (2c, 2d); Group III (2e, 2f); Group IV (2g, 2h). Primordial (α), primary (β), secondary (γ), and antral (δ) follicles appear normal in the control group (2a, 2b). Zona pellucida (ZP) is observed around the secondary follicle (γ). Cortex (C) and medulla (M) are identified with normal vasculature (arrows) (2a, 2b). The I/R group shows vascular dilatation (arrows), atretic follicles (AF) with degenerated ZP, apoptotic bodies (asterisk), and primordial follicles with edema (α) (2c, 2d). I/R+NIF group shows vascular dilatation (arrows) however follicles appeared close to the control group (2e, 2f) with well-vascularized structures within theca interna of antral follicles (δ) (2f). There were no AF. I/R+RSV group showed normal Primordial (α), and primary (β) follicles (2g) but, primary (β) and antral follicles (δ) were atretic (2h)

*H&E: Hematoxylin and eosin, NIF: Nifedipine, RSV: Resveratrol, I/R: Ischemia-reperfusion*

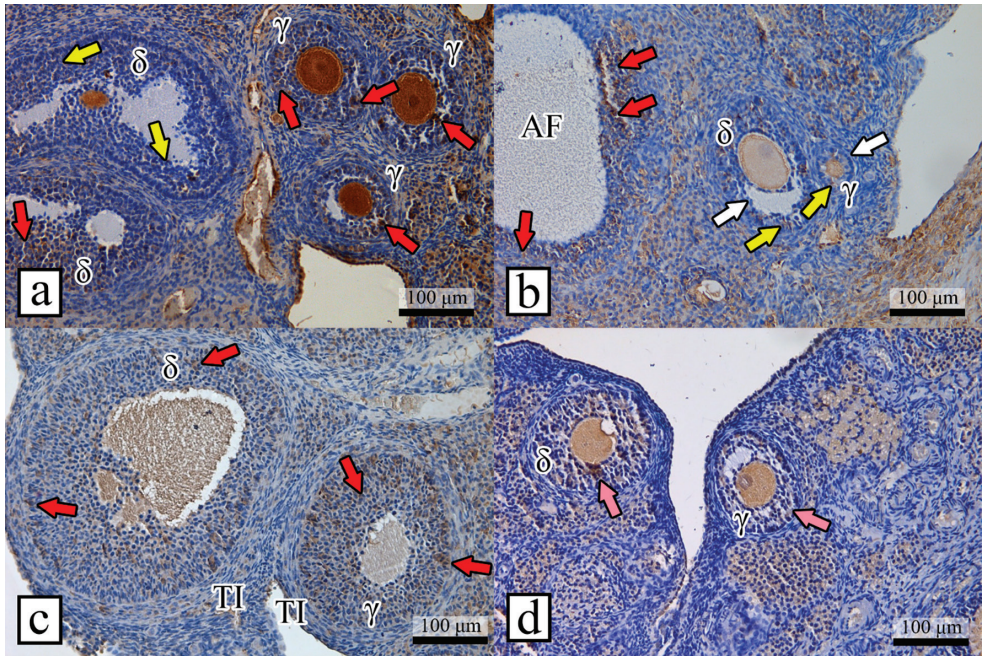


**Figure 3.** Representative micrographs of AMH-immunostained ovarian tissue sections captured under 200 x magnification, in all groups: Group I (3a); Group II (3b); Group III (3c); Group IV (3d). Secondary follicles (γ) show high (red arrows), antral follicles (δ) show low (yellow arrows) and high (red arrows) AMH reactivity in the control group (3a). The I/R group showed markedly decreased AMH reactivity from low (yellow arrows) to negative (white arrows) with a low number of reactive secondary (γ) and antral (δ) follicles as well as atretic follicles with high immunoreactivity (3b). The I/R+NIF group was similar to the control group with high reactivity (red arrows) in secondary (γ) and small antral follicles (δ), with more reactivity closer to theca interna (3c). Although the I/R+RSV group showed better immunoreactivity (pink arrows) than the I/R group in secondary (γ) and antral follicles (δ), it remained lower than the control group (3d)

*AMH: Anti-müllerian hormone, NIF: Nifedipine, RSV: Resveratrol, I/R: Ischemia-reperfusion*

I/R injury and reported the primary, antral, graafian, and atretic follicle count were higher, indicating a positive effect. Santa-Helena et al.[24], also observed that nifedipine reduced reactive oxygen radicals and increased the viability of cardiomyoblasts after I/R injury. This indicates that nifedipine may have a role against I/R injury. In another study on the same topic, the effect of enoxaparin was investigated, and although primordial, preantral, corpus luteum and atretic follicle counts were not found to be different, the number of small and large antral follicles was significantly lower in the torsion/detorsion group compared to the control[25]. We found that the nifedipine-treated group had a higher secondary follicle count than groups 2 and 4, a finding which warrants further investigation in clinical studies.

We performed AMH and VEGFR-2 immunostaining in ovarian tissues. Parlakgumus et al.[13] studied atorvastatin and expression of AMH and VEGF-A in I/R damaged ovaries, and they found that AMH was higher in the torsion/detorsion + atorvastatin group. The expression of AMH originates in granulosa cells

**Table 3.** Comparison of groups for primordial and secondary follicles

| | Primordial follicle | Secondary follicle |
|---|---|---|
| Group 1/Group 2 | 0.014 | 0.05 |
| Group 1/Group 3 | 0.123 | 0.41 |
| Group 1/Group 4 | 0.092 | 0.078 |
| Group 2/Group 3 | 0.273 | **0.013** |
| Group 2/Group 4 | 0.213 | 0.374 |
| Group 3/Group 4 | 1 | **0.017** |

Independent t-test. P<0.05 was considered statistically significant

**Table 4.** Evaluation of TAS, TOS and OSI (±: standard deviation)

| | Group 1 (SD) | Group 2 (SD) | Group 3 (SD) | Group 4 (SD) | p-value |
|---|---|---|---|---|---|
| TAS (mmol/L) | 0.37 (0.17) | 0.4 (0.12) | 0.58 (0.13) | 0.45 (0.14) | 0.094 |
| TOS (µmol/L) | 3.4 (1.3) | 5.6 (5.1) | 9.0 (3.5) | 8.6 (6.7) | 0.162 |
| OSI | 1.1 (0.8) | 1.3 (0.8) | 1.6 (0.7) | 1.7 (1.1) | 0.611 |

SD: Standard deviation, TAS: Total antioxidant status, TOS: Total oxidant status, OSI: Oxidative stress index

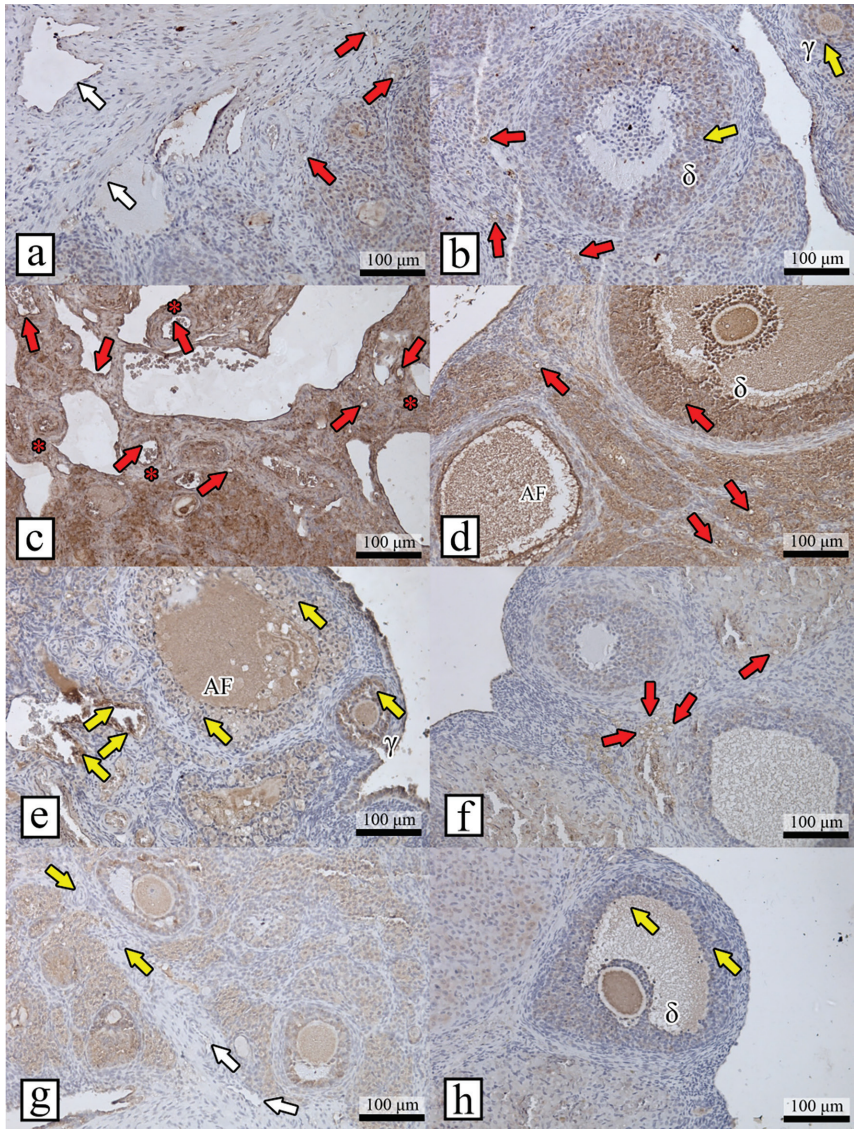


**Figure 4.** Representative micrographs of VEGFR-2-immunostained ovarian tissue sections captured under 200 x magnification, in all groups: Group I (4a, 4b); Group II (4c, 4d); Group III (4e, 4f); Group IV (4g, 4h). No uptake was detected around large vessel endothelial cells (white arrows), but capillary uptake was present (yellow arrows) in endothelial cells in the medulla in the control group (4a). Viewing the cortex, secondary (γ) and antral (δ) follicles in this section showed weak uptake (yellow arrows), while capillary here showed similar uptake (red arrows) (4b). The I/R group was different with tissue-wide, strong uptake in the medulla, with capillary as well as large vessel (red arrows) endothelial uptake, and high uptake in smooth muscle cells (asterisk) (4c). Cortex also showed high reactivity around capillary endothelial cells, antral (δ), and atretic follicles (AF) (red arrows) (4d). The I/R+NIF group showed medium uptake, closer to the I/R group rather than the control, with medullary uptake (yellow arrows) around large vessel endothelial cells, granulosa of secondary (γ) and AF (4e) and high capillary uptake (red arrows) in the cortex (4f). The I/R+RSV group showed a potentially better effect against oxidative stress than the previous group with negative (white arrows) to weak (yellow arrows) uptake in both large vessels and capillaries in the medulla (4g) as well as weak (yellow arrows) uptake similar to the control group in antral follicles (δ) (4h)

*VEGFR-2: Vascular endothelial growth factor receptor 2, NIF: Nifedipine, RSV: Resveratrol, I/R: Ischemia-reperfusion*

of early antral follicles, and the follicles expressing the most AMH are preantral and small antral follicles[26,27]. Ischemia is a factor that reduces AMH expression. Cells expressing higher AMH are thought to have higher proliferative and steroidogenic activity[28]. In our study, the staining of preantral and antral follicles for AMH was weak in groups II and IV, although it was close to control in the nifedipine-treated group. This suggests that nifedipine may preserve the proliferative capacity of cells.

Hypoxia is one of the best stimulators of angiogenesis, thus increased expression of VEGF receptors during I/R can be expected. Kanellis et al.[29] showed that after renal I/R, VEGFR-2 immunostaining surged in renal tissue. In our study,

although the staining of VEGFR-2 was increased in group 2, it was decreased in the resveratrol-treated group. The lower VEGF receptor level in group 4 suggests that resveratrol can help in tolerating oxidative stress and its effects. Evidence suggests that calcium channel blockers may increase VEGF concentration[30,31]. Our findings indicate that the staining of VEGFR-2 in the nifedipine group was similar to that in the I/R group alone. Therefore, it is suggested that nifedipine has no significant effect on VEGF.

Oxidative stress was similar among groups in the current study. Gungor et al.[32] evaluated the possible beneficial effects of a clinically available omega-3 fatty acid emulsion on I/R injury by measuring TAS, TOS, and OSI, and they did not find any positive effect. Although resveratrol and nifedipine were shown to have antioxidant effects in several studies, we did not observe any evidence of this effect in our study[33].

### Study Limitations

There are several limitations that should be noted. First, we performed torsion for only 3 hours. However, in clinical ovarian torsion scenarios, the exact duration from torsion to detorsion is often unknown. As the torsion duration increases, the damage to the ovary might become irreparable. Moreover, we administered the agents at doses used in previously published studies and have not tested other doses, which may have allowed more optimal dosing regimens.

## Conclusion

Although nifedipine and resveratrol did not demonstrate a concrete protective effect on every parameter evaluating I/R injury after ovarian torsion/detorsion, increased immunohistochemical AMH expression and secondary follicle count in the nifedipine treated group, and decreased VEGFR-2 expression in the resveratrol treated group, can be regarded as indicators of their potential.

### Ethics

**Ethics Committee Approval:** The protocol was reviewed and approved by the Gazi University Animal Experiments Local Ethics Committee of animal studies (approval number: G.Ü.ET-20.034, date: 09.07.2020).

**Informed Consent:** Not necessary.

### Footnotes

### Authorship Contributions

Surgical and Medical Practices: A.S., A.A.E.S., G.T.K., C.K., E.D., M.F.C.A., M.E., A.E., M.Ö., Concept: A.S., A.A.E.S., G.T.K., C.K., E.D., M.F.C.A., M.E., A.E., M.Ö., Design: A.S., A.A.E.S., G.T.K., C.K., E.D., M.F.C.A., M.E., A.E., M.Ö., Data Collection or Processing: A.S., A.A.E.S., G.T.K., C.K., E.D., M.F.C.A., M.E., A.E., M.Ö., Analysis or Interpretation: A.S., A.A.E.S., G.T.K., C.K., E.D., M.F.C.A., M.E., A.E., M.Ö., Literature Search: A.S., A.A.E.S., G.T.K., C.K., E.D., M.F.C.A., M.E., A.E., M.Ö., Writing: A.S., A.A.E.S., G.T.K., C.K., E.D., M.F.C.A., M.E., A.E., M.Ö.

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

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*Review / Derleme*

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# The role of secretome in restoring ovarian function: A systematic review and meta-analysis of *in vivo* studies in mice with premature ovarian insufficiency

## *Yumurtalık fonksiyonunun geri kazandırılmasında sekretomun rolü: Erken yumurtalık yetmezliği olan farelerde in vivo çalışmaların sistematik bir incelemesi ve meta-analizi*

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### Abstract

To evaluate the efficacy of secretomes in restoring ovarian function in premature ovarian insufficiency (POI) in a mouse model, researchers emphasizing their potential as a novel, cell-free therapy. This systematic review and meta-analysis followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2020 guidelines and included studies from four databases through December 2023. The bias risk was assessed using the tool for animal studies, which is Systematic Review Centre for Laboratory Animal Experimentation risk-of-bias. Outcomes, including the hormonal levels of estradiol (E2), anti-müllerian hormone (AMH), and follicle-stimulating hormone (FSH) were analyzed, with statistical comparisons made between the secretome-treated and control groups. Four studies encompassing sixty mice were included. The meta-analysis showed a significant increase in E2 levels in the secretome group [mean difference (MD)=37.45, 95% confidence interval (CI): 5.78 to 69.11; p=0.02]. No significant difference in AMH levels was observed; however, a sensitivity analysis resulted in the difference becoming statistically significant (MD=1.83, 95% CI: 0.95 to 2.71; p<0.0001). Moreover, the analysis revealed a significant reduction in FSH levels in the secretome group (MD=-36.80, 95% CI: -61.91 to -11.69; p=0.004) even after the sensitivity analysis. Our findings demonstrated enhanced outcomes with secretome therapy in the management of POI. Further research, particularly involving human subjects, is necessary to validate these findings.

**Keywords:** Hormones, ovarian function tests, premature ovarian failure, primary ovarian insufficiency, secretome

### Öz

Bir fare modelinde prematüre yumurtalık yetmezliğinde (POI) sekretomların yumurtalık fonksiyonunu geri kazanmadaki etkinliğini değerlendirmek için araştırmacılar, yeni, hücresiz bir tedavi olarak potansiyellerini vurgulamaktadır. Bu sistematik inceleme ve meta-analiz, Sistematik İncelemeler ve Meta-Analizler için Tercih Edilen Raporlama Öğeleri 2020 yönergelerini izlemiş ve Aralık 2023'e kadar dört veri tabanından çalışmaları içermiştir. Yanlılık riski, Laboratuvar Hayvan Deneyleri için Sistematik İnceleme Merkezi yanlılık riski olan hayvan çalışmaları aracı kullanılarak değerlendirilmiştir. Östradiol (E2), anti-müllerian hormon (AMH) ve folikül uyarıcı hormon (FSH) hormonal seviyeleri de dahil olmak üzere sonuçlar, sekretomla tedavi edilen ve kontrol grupları arasında yapılan istatistiksel karşılaştırmalarla analiz edilmiştir. Altmış fareyi kapsayan dört çalışma dahil edilmiştir. Meta-analiz, sekretom grubunda E2 seviyelerinde önemli bir artış olduğunu göstermiştir [ortalama fark (OF) = 37,45, %95 güven aralığı (GA): 5,78 ila 69,11; p=0,02]. AMH düzeylerinde anlamlı bir fark gözlenmemiştir; ancak duyarlılık analizi sonucunda fark istatistiksel olarak anlamlı hale gelmiştir (OF=1,83, %95 GA: 0,95

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ila 2,71; p<0,0001). Ayrıca, analiz, duyarlılık analizinden sonra bile sekretom grubunda FSH seviyelerinde önemli bir düşüş olduğunu ortaya koymuştur (MD=-36,80, %95 CI: -61,91 ila -11,69; p=0,004). Bulgularımız, POI tedavisinde sekretom tedavisi ile daha iyi sonuçlar elde edildiğini göstermiştir. Bu bulguları doğrulamak için özellikle insan denekleri içeren daha fazla araştırma yapılması gerekmektedir.

**Anahtar Kelimeler:** Hormonlar, yumurtalık fonksiyon testleri, prematüre yumurtalık yetmezliği, primer yumurtalık yetmezliği, sekretom

## Introduction

Premature ovarian insufficiency (POI), also referred to as premature ovarian failure (POF), occurs when ovarian function stops before the age of 40, and is widely known as early menopause. This condition is a primary factor of female infertility under 40, impacting an estimated 3.7% of women[1]. The prevalence of POI varies among different ethnic groups, and approximately 15% of cases are associated with a family history, indicating a potential genetic basis for the disorder[2].

The current typical therapeutic approach for POI involves taking continuous hormone replacement therapy (HRT) to alleviate estrogen deficiency symptoms and reduce the risks of osteoporosis and cardiovascular disease. This treatment is generally recommended until the age of natural menopause. However, HRT works only by mimicking the effect of hormones, which does not restore ovarian function or fertility[3]. Recently, the secretome, which consists of proteins including extracellular matrix proteins, proteins shed from the cell membrane, and vesicle proteins, has emerged as a promising treatment for POI. It shows therapeutic effects such as promoting the formation of new blood vessels, known as angiogenesis, reducing inflammation, and evading immune responses, potentially improving the restoration of ovarian function and fertility[4].

Due to the comparable features of the estrous cycle in mice and the human menstrual cycle, mouse models serve as crucial tools for investigating POI pathogenesis and advancing therapeutic strategies[5]. Previous meta-analyses, such as those conducted by Hu et al.[5] and Kim et al.[6], have also utilized POI mouse models, highlighting the effectiveness of stem cell therapy in restoring fertility in POI models and patients, while a meta-analysis by Luo et al.[6-8] showed that stem cell-derived extracellular vesicles (EVs) are safe and effective in treating animal models of POI, with promising potential to enhance fertility outcomes.

Stem cells, EVs, and secretomes are distinct regenerative approaches with unique characteristics. Stem cells rely on cellular transplantation, while EVs act as carriers of cellular signals. On the other hand, secretomes consist of bioactive molecules such as growth factors and cytokines; they offer a broader, cell-free therapeutic option with reduced risks of immune rejection and are not limited to vesicular structures only[9]. While meta-analyses have explored stem cells and EVs, the role of secretomes in treating POI has yet to be systematically analyzed. This systematic review presents the first meta-analysis of *in vivo* studies regarding the efficacy of secretomes in restoring ovarian function in mice with POI, highlighting their therapeutic potential.

## Materials and Methods

### Literature Searching

This meta-analysis was performed using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2020 guidelines[8]. The meta-analysis was based on published articles and therefore did not require ethical approval. This research was conducted across four databases (Science Direct, Sage Journals, PubMed, and ProQuest) through December 2024. The search strategy used essential keywords and Medical Subject Headings (MeSH) terms such as “secretome”, “premature ovarian insufficiency”, “primary ovarian insufficiency”, and “premature ovarian failure”. Boolean operators (AND/OR) were utilized to refine search results.

### Study Selection

Animal studies focusing on the use of secretome in POI mouse models were included. Studies were excluded if they did not involve POI mouse models or failed to assess hormonal levels, including estradiol (E2), anti-müllerian hormone (AMH), or follicle-stimulating hormone (FSH), as outcomes. All records were imported into EndNote X9, where duplicates were removed using the software’s identification tool. Titles, abstracts, and full-text articles were reviewed by two reviewers independently, with any disagreements resolved through consultation with an additional author.

### Data Extraction

Three investigators were involved in the data extraction, which was performed independently. Any difference was resolved by consensus or by consulting an additional author. Extracted data included study characteristics (authors, publication year, mice model experiment, and stem cell-derived secretome). Hormonal levels (E2, AMH, and FSH) were evaluated for each study, with numerical data extracted from graphs using WebPlotDigitizer, followed by statistical analysis to evaluate the outcomes. The results include a grouped analysis of E2, AMH, and FSH levels comparing the untreated or control group and the secretome-treated group.

### Risk of Bias Analysis

The risk of bias was independently assessed by two investigators using the Systematic Review Centre for Laboratory Animal Experimentation risk-of-bias tool for animal studies[9]. This evaluation addressed ten key domains: (1) generation of sequence, (2) characteristics of baseline, (3) concealment of allocation, (4) random housing, (5) blind caregivers and investigators, (6) random outcome assessment, (7) outcome

assessment blinded to assessors, (8) incomplete data outcome, (9) selective outcome reporting, and (10) other bias sources. Each domain was categorized as "Yes" if low risk of bias, "No" if high risk of bias, or "Unclear" if the study text provided insufficient information to make a definitive judgment. Any discrepancies were discussed and resolved through consultation with an additional reviewer.

### Statistical Analysis

E2, AMH, and FSH levels were compared between the secretome-treated group and the control group for analysis. Heterogeneity was evaluated using the $I^2$ statistic, with a p-value <0.05 considered indicative of substantial heterogeneity. Sensitivity analyses, excluding studies with potential bias, were conducted when significant heterogeneity was detected. All statistical analyses were performed using Cochrane Review Manager software version 5.4.

## Results

Two hundred forty-seven articles were gathered from the literature search, and 4 met the eligibility criteria to be analyzed, as depicted in Figure 1. Between 2021 and 2024, those studies involving a total of 60 mice were published.

All studies were assessed to have a low risk of bias in sequence generation, attrition bias, reporting bias, and other sources of bias. However, all studies showed an unclear risk of bias regarding allocation concealment, performance bias, and detection bias related to blinding. For baseline characteristics, two studies [Park et al.[10] and Le et al.[13]] had a low risk of bias, while the other two [Zhang et al.[11] and Nabil Salama et al.[12]] had an unclear risk. Regarding random outcome assessment, two studies [Park et al.[10] and Le et al.[13]] demonstrated a low risk of bias, whereas the remaining two [Nabil Salama et al.[12] and Le et al.[13]] were categorized as indeterminate risk (Table 1).

The meta-analysis showed an increase in E2 levels in the secretome group, which is significant when compared to the control group, [mean difference (MD) = 37.45, 95% confidence interval (CI): 5.78 to 69.11; p=0.02], with considerable heterogeneity ($I^2$=98%) (Figure 2). Excluding two studies in the sensitivity analysis strengthened the reliability of the findings (Figure 3). After exclusion, E2 levels remained significantly higher in the secretome group than the control group (MD=34.45, 95% CI: 26.95 to 41.95; p<0.0001), with a marked reduction in heterogeneity ($I^2$=29%). This analysis affirms that the link between secretome intervention and elevated E2 levels remains statistically significant, even after accounting for potential biases, thereby further supporting the consistency and robustness of the results across studies.

The pooled analysis of the included studies revealed no significant MD in AMH levels between the secretome group and the control group (MD=3.71, 95% CI: -0.55 to 7.97; p=0.09), with a high degree of heterogeneity observed ($I^2$=98%) (Figure 4). However, the sensitivity analysis, which excluded one study, demonstrated a substantial reduction in heterogeneity to 0%. Furthermore, after excluding this study, the difference between the groups became significant based on the statistics, with an MD of 1.83 (95% CI: 0.95 to 2.71; p<0.0001) (Figure 5).

The pooled analysis of all included studies showed that FSH levels in the secretome group reduce in significant amount if compared with the control group (MD=-36.80, 95% CI: -61.91 to -11.69; p=0.004) (Figure 6). The sensitivity analysis, which excluded one study, confirms the robustness of the findings (Figure 7). FSH levels were significantly reduced in the secretome group compared to the control group (MD=-25.58, 95% CI: -38.35 to -12.81; p<0.0001), with a decrease in heterogeneity ($I^2$=62%). This analysis indicates that the association between secretome intervention and reduced FSH levels remains statistically significant, even after accounting for potential bias, thereby reinforcing the strength and consistency of the findings across studies.

## Discussion

This meta-analysis evaluates the potential of secretome therapy in restoring ovarian function by assessing its impact on E2, AMH, and FSH levels as the main parameters of female hormones regarding fertility. The results demonstrate that the secretome group exhibited significantly increased E2 and AMH levels and decreased FSH levels compared to the control group, as supported by sensitivity analyses. For E2, the sensitivity

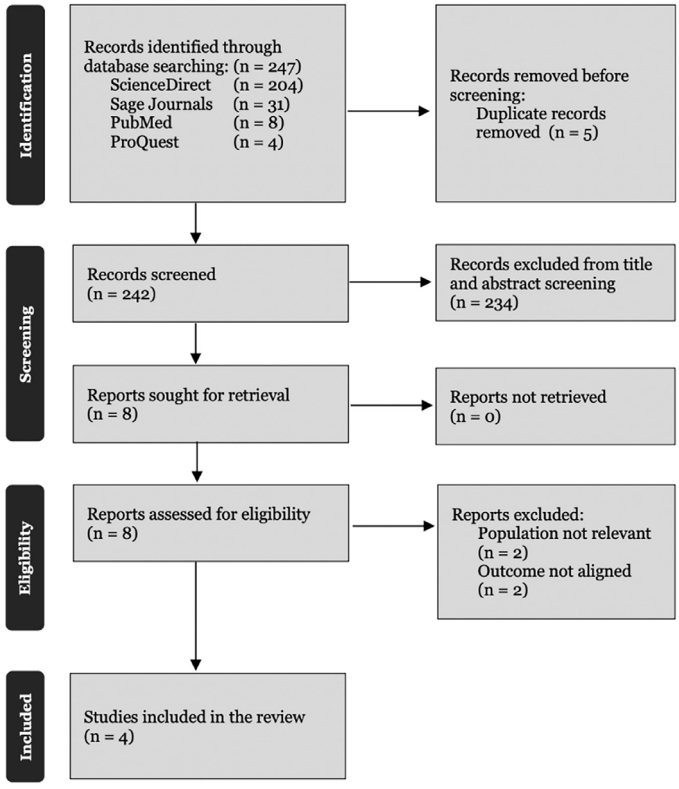


**Figure 1.** PRISMA flow diagram depicting study selection

*PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses*

**Table 1.** Risk of bias assessment using SYRCLE risk of bias tool for animal studies

| Study | Selection bias | | | Performance bias | | Detection bias | | Attrition bias | Reporting bias | Other bias |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sequence generation | Baseline characteristics | Allocation concealment | Random housing | Blinding | Random outcome assessment | Blinding | Incomplete outcome data | Selective outcome data | Other sources bias |
| Park et al. 2021[10] | Yes | Yes | Unclear | Unclear | Unclear | Yes | Unclear | Yes | Yes | Yes |
| Zhang et al. 2021[11] | Yes | Unclear | Unclear | Unclear | Unclear | Yes | Unclear | Yes | Yes | Yes |
| Salama et al. 2023[12] | Yes | Unclear | Unclear | Unclear | Unclear | Unclear | Unclear | Yes | Yes | Yes |
| Le et al. 2024[13] | Yes | Yes | Unclear | Unclear | Unclear | Unclear | Unclear | Yes | Yes | Yes |

SYRCLE: Systematic Review Centre for Laboratory Animal Experimentation

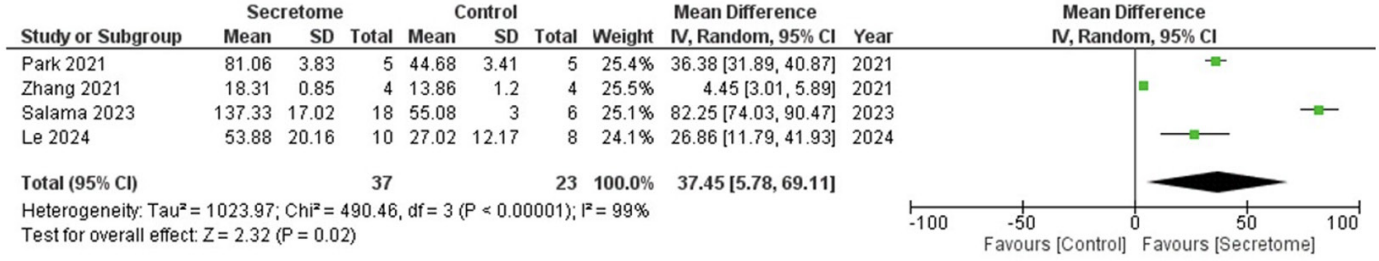


**Figure 2.** Forest plot: Pooled analysis of E2 levels between the secretome group and the control group

*E2: Estradiol, SD: Standard deviation, CI: Confidence interval, IV: Interval variable*

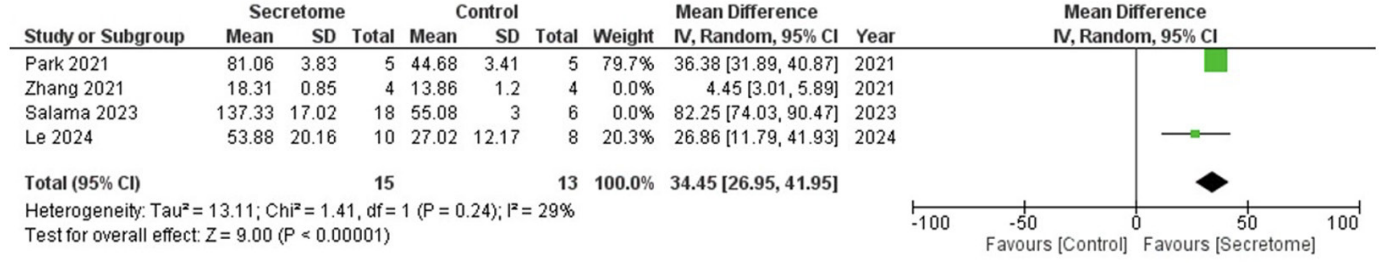


**Figure 3.** Forest plot: Sensitivity analysis of E2 levels between the secretome group and control group

*E2: Estradiol, SD: Standard deviation, CI: Confidence interval, IV: Interval variable*

analysis excluded the study by Zhang et al.[11] due to notable variations in baseline E2 levels among the mice. Additionally, sensitivity analyses for E2, AMH, and FSH excluded the study by Nabil Salama et al.[12] as it involved distinct baseline characteristics, particularly a substantial age discrepancy among the mice, as shown in Table 2.

E2 is a steroid hormone that is related to the ovarian follicle development and egg maturity. Elevated E2 levels in larger follicles play a crucial role in the selection of a dominant follicle and the induction of the LH surge required for ovulation. However, POI is associated with disrupted folliculogenesis, such as diminished follicular recruitment and dysfunction and premature follicular atresia. As a result, E2 serves as a critical marker of follicle quality in folliculogenesis and holds a potential role for restoring ovarian function in patients with POI[14]. Our study showed the E2 levels in the secretome group increased by a significant amount compared to the control. The results of this analysis further validate the consistency and robustness of the link between secretome intervention and elevated E2 levels, demonstrating that the relationship holds across multiple

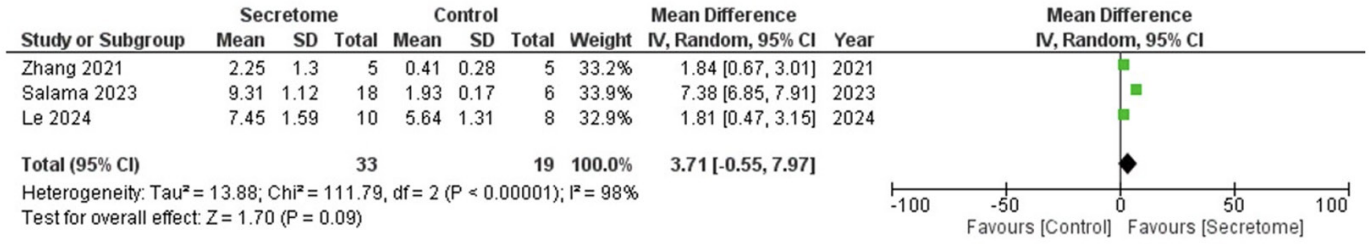


**Figure 4.** Forest plot: Pooled analysis of AMH levels between the secretome group and the control group

*AMH: Anti-müllerian hormone, SD: Standard deviation, CI: Confidence interval, IV: Interval variable*

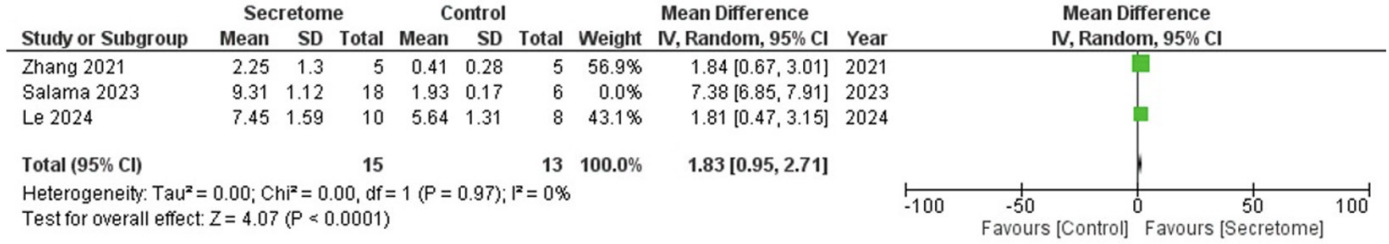


**Figure 5.** Forest plot: Sensitivity analysis of AMH levels between the secretome group and control group

*AMH: Anti-müllerian hormone, SD: Standard deviation, CI: Confidence interval, IV: Interval variable*

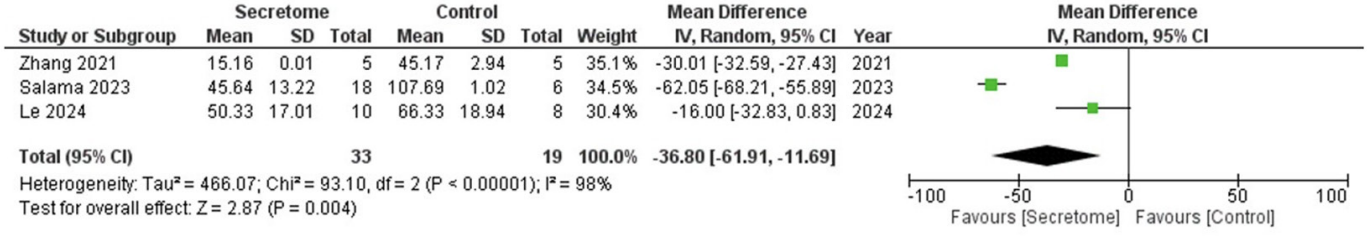


**Figure 6.** Forest plot: Pooled analysis of FSH levels between the secretome group and the control group

*FSH: Follicle-stimulating hormone, SD: Standard deviation, CI: Confidence interval, IV: Interval variable*

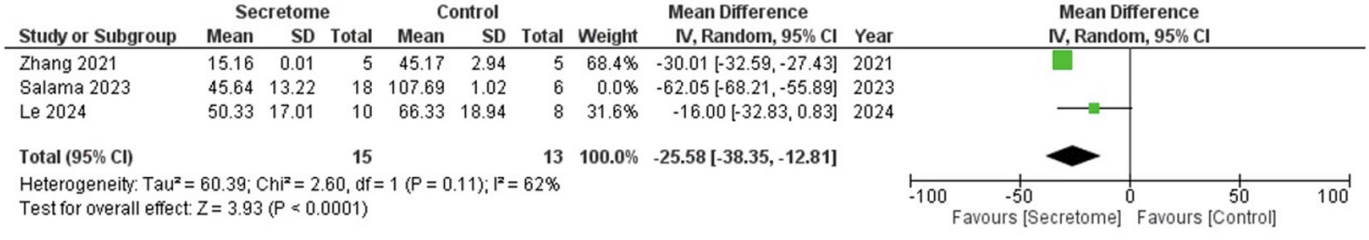


**Figure 7.** Forest plot: Sensitivity analysis of FSH levels between the secretome group and control group

*FSH: Follicle-stimulating hormone, SD: Standard deviation, CI: Confidence interval, IV: Interval variable*

studies. This strengthens the evidence for the reliability of the secretome effect in developing targeted therapeutic strategies in POI patients.

In this study, we observed a significant increase in AMH levels in the secretome-treated group compared to the control group, suggesting that secretomes may exert a protective effect on ovarian reserve. Granulosa cells of the growing follicles secrete AMH, which is a glycoprotein and has been shown an important role in maintaining ovarian reserve in both murine models and humans[11,15]. As a member of the transforming growth factor-β superfamily, AMH serves as a pivotal growth factor in the ovary by inhibiting primordial follicles recruitment and modulating

**Table 2.** Characteristic of included studies

| Study | Mice model experiment | | | | Stem cell-derived secretome |
|---|---|---|---|---|---|
| | Model | Type | Age | Drugs used | |
| Park et al. 2021[10] | Chemotherapy-induced POI mice model | Female C57BL/6 mice | 6 weeks old | Busulfan, Cyclophosphamide | Human BM-MSC secretome |
| Zhang et al. 2021[11] | Chemotherapy-induced POI mice model | Female Sprague-Dawley rats | 5-7 weeks old | Cisplatin | ORP secretome by embedding HUC-MSC |
| Salama et al. 2023[12] | Chemotherapy-induced POI mice model | Female C57BL/6 mice | 2-3 months old | Cisplatin | Human BM-MSC secretome |
| Le et al. 2024[13] | Chemotherapy-induced POI mice model | Female C57BL/6J mice | 7 weeks old | Cyclophosphamide | Human placenta choriodecidual membrane tissues ER+pc MSC secretome |

BM: Bone marrow, ER+pc: Estrogen receptor positive, HUC: Human umbilical cord, MSC: Mesenchymal stem cell, ORP: Ovarian regenerative patch, POI: Premature ovarian insufficiency

follicular sensitivity to FSH during folliculogenesis[16] and diminishing the chemotherapy-induced ovarian dysfunction, thereby reducing the risk of POI[17]. These findings highlight the importance of secretomes, as one of the therapeutic strategies promising for the management of POI.

Our study also revealed a decrease in FSH levels in the group that was treated with secretome compared to the control group. This reduction is likely due to the effect of secretomy by promoting angiogenesis, facilitating tissue repair, and supporting follicular survival in damaged ovaries. By alleviating chronic inflammation that causes disruption to ovarian function and disrupts hormonal feedback mechanisms, the secretome has the benefit of restoring hormonal homeostasis. The vascular endothelial growth factor and insulin-like growth factor are the growth factors present in the secretome that stimulate follicular development, while their anti-apoptotic properties protect ovarian follicles. Collectively, these mechanisms enhance ovarian function, re-establishing effective negative feedback to the pituitary gland and subsequently reducing FSH production[18-20]. Based on all the parameters evaluated in this meta-analysis, it is shown that secretome has a positive impact on the management of POF, especially regarding the fertility issue, and can become one of the options rather than HRT.

### Study Limitations

This systematic review and meta-analysis has several limitations. The small sample sizes in the included studies are not optimal for the generalizability of our findings. Moreover, variations in baseline characteristics and the different types of stem cell-derived secretome used may affect the results. Future studies with larger sample sizes and methods that are further standardized are needed to strengthen the evidence and enable updates to this meta-analysis.

## Conclusion

Our study found that secretome therapy significantly improved outcomes in the management of POI. Specifically, the therapy resulted in increased E2 and AMH levels and decreased the level of FSH. These hormonal changes are indicative of restored ovarian function, suggesting that secretome therapy may help address the hormonal imbalance characteristic of POI. Additionally, our findings demonstrate improvements in other symptoms associated with POI, further supporting the potential of secretome therapy as a promising treatment. However, while these results are encouraging, further studies, particularly those involving human subjects, are essential to fully evaluate the efficacy and safety of secretome therapy in this context. Large-scale, well-designed clinical investigations are needed to evaluate these findings, especially the long-term outcomes, and establish clear guidelines for their clinical application.

### Footnotes

### Authorship Contributions

Surgical and Medical Practices: S.K., I.G.E.W., I.N.G.B., Concept: S.K., I.G.E.W., I.W.P.S.Y., Design: S.K., I.G.E.W., Data Collection or Processing: S.K., I.G.E.W., I.N.G.B., Analysis or Interpretation: S.K., I.G.E.W., I.W.P.S.Y., Literature Search: S.K., I.G.E.W., I.N.G.B., Writing: S.K., I.W.P.S.Y.

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

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*Review / Derleme*

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# Risk of cardiovascular and cerebrovascular events in polycystic ovarian syndrome women: An updated meta-analysis of cohort studies

## *Polikistik over sendromlu kadınlarda kardiyovasküler ve serebrovasküler olay riski: Kohort çalışmalarının güncellenmiş meta-analizi*

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**Abstract**

Polycystic ovary syndrome (PCOS), affecting 5-10% of reproductive-aged women, is linked to metabolic disturbances such as insulin resistance, obesity, and lipid imbalance, which may elevate cardiovascular disease (CVD) risk. The relationship between PCOS and clinical cardiovascular events remains unclear. This meta-analysis evaluates the association between PCOS and cardiovascular and cerebrovascular events, including myocardial infarction (MI), stroke, ischemic heart disease (IHD), and overall CVD. We conducted a systematic review and meta-analysis of observational cohort studies published up to August 2024. Studies investigating the association between PCOS and cardiovascular or cerebrovascular events were included. Hazard ratios (HR) were used to assess mortality risk, while odds ratios (OR) evaluated CVD incidence. Statistical analyses were performed using STATA software, with publication bias assessed via funnel plots. Nineteen cohort studies, involving 1,222,912 participants, were analyzed. Women with PCOS had a significantly higher risk of stroke [OR: 1.89, 95% confidence interval (CI): 1.22-2.55]. However, no significant associations were found between PCOS and overall CVD (HR: 1.80, 95% CI: 5.43-9.04), MI (HR: 2.68, 95% CI: 0.69-4.82), or IHD (HR: 2.68, 95% CI: 0.69-4.67). Additionally, there was no significant increase in cardiovascular or all-cause mortality. This meta-analysis highlights that women with PCOS are at an increased risk of stroke, but no conclusive evidence links PCOS to other cardiovascular outcomes or mortality. Clinicians should prioritize stroke prevention in this population. Further large-scale, long-term studies are needed to clarify the cardiovascular risks associated with PCOS.

**Keywords:** Polycystic ovary syndrome, cardiovascular diseases, cerebrovascular disorders, meta-analysis

**Öz**

Üreme çağındaki kadınların %5-10'unu etkileyen polikistik over sendromu (PKOS), insülin direnci, obezite ve lipid dengesizliği gibi metabolik bozukluklarla bağlantılıdır ve bu da kardiyovasküler hastalık (KVH) riskini artırabilir. PKOS ile klinik kardiyovasküler olaylar arasındaki ilişki henüz net değildir. Bu meta-analiz, PKOS ile miyokard enfarktüsü (MI), inme, iskemik kalp hastalığı (İKH) ve genel KVH dahil olmak üzere kardiyovasküler ve serebrovasküler olaylar arasındaki ilişkiyi değerlendirmektedir. Ağustos 2024'e kadar yayınlanmış gözlemsel kohort çalışmalarının sistematik bir incelemesini ve meta-analizini gerçekleştirdik. PKOS ile kardiyovasküler veya serebrovasküler olaylar arasındaki ilişkiyi araştıran çalışmalar dahil edildi. Tehlike oranları (HR) ölüm riskini

**PRECIS:** Women with polycystic ovary syndrome (PCOS) have a significantly higher risk of stroke but no conclusive evidence links PCOS to other cardiovascular events or mortality.

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değerlendirmek için kullanılırken, olasılık oranları (OR) KVH insidansını değerlendirdi. İstatistiksel analizler STATA yazılımı kullanılarak gerçekleştirildi ve yayında taraf tutma huni grafikleri aracılığıyla değerlendirildi. Bu meta-analizde 1.222.912 katılımcıyı içeren on dokuz kohort çalışması analiz edildi. PKOS'lu kadınlarda inme riski önemli ölçüde daha yüksekti [OR: 1,89, %95 güven aralığı (GA): 1,22-2,55]. Ancak PKOS ile genel KVH (HR: 1,80, %95 GA: 5,43-9,04), MI (HR: 2,68, %95 GA: 0,69-4,82) veya İKH (HR: 2,68, %95 GA: 0,69-4,67) arasında önemli bir ilişki bulunamadı. Ek olarak, kardiyovasküler veya tüm nedenlere bağlı ölüm oranında önemli bir artış olmadı. Bu meta-analiz, PKOS'li kadınların inme geçirme riskinin arttığını vurgulamaktadır, ancak PKOS'yi diğer kardiyovasküler sonuçlar veya ölüm oranıyla ilişkilendiren kesin bir kanıt yoktur. Klinisyenler bu popülasyonda inmeyi önlemeyi önceliklendirmelidir. PKOS ile ilişkili kardiyovasküler riskleri açıklığa kavuşturmak için daha fazla büyük ölçekli, uzun vadeli çalışmalara ihtiyaç vardır.

**Anahtar Kelimeler:** Polikistik over sendromu, kardiyovasküler hastalıklar, serebrovasküler bozukluklar, meta-analiz

## Introduction

An endocrine condition, known as polycystic ovarian syndrome (PCOS), affects 5-10% of women who are of reproductive age and is characterized by common phenotypic and clinical symptoms[1,2]. It is brought on by insulin resistance (IR), follicular dysplasia, and hyperandrogenism, which collectively contribute to manifestations such as obesity, infertility, irregular menstruation, and hyperandrogenemia[3,4]. Metabolic disturbances are common in PCOS patients and are easily associated with other metabolic synthesis disorders, obesity, hypertension, diabetes, and disorders of lipid metabolism[5]. Therefore, there is a considerable rise in the risk of cardiovascular disease (CVD) in PCOS patients due to the increased risk of atherosclerosis, as indicated in citation[6].

Since PCOS frequently exhibits IR, altered glucose regulation, dyslipidemia, high blood pressure (BP), and obesity as early as young adulthood or even childhood, women with PCOS are exposed to conventional cardiovascular risk factors over an extended period. Nevertheless, data regarding the relationship between the presence of numerous CVD risk factors and an increased risk of CVD events in PCOS-affected women are inconsistent[7,8]. These discrepancies may stem from variations in the diagnostic criteria for PCOS, differences in metabolic profiles, definitions of cardiovascular outcomes, or methodological limitations such as small sample sizes and study design differences. Notably, individuals meeting the National Institutes of Health (NIH) criteria for PCOS tend to exhibit more severe metabolic disturbances and may carry a greater cardiovascular risk than those diagnosed under the broader Rotterdam criteria. We also are not certain if women who have phenotypes C and D, which are non-NIH PCOS phenotypes, are more likely to experience CVD events[9].

The correlation between PCOS and a higher long-term risk of CVD events is still up for debate, despite mounting data linking PCOS to CVD risk factors. It is unclear if established risk factors mitigate the relationship between PCOS and CVD, or if PCOS is a separate risk factor[10]. Notably, many participants in earlier studies were overweight or obese, highlighting the substantial impact of excess weight on conventional cardiovascular risk markers[11]. Some previous meta-analyses have suggested an increased incidence of cardiovascular outcomes-such as coronary artery disease and cerebrovascular conditions-among women with PCOS[12]. Women from East Asian populations with PCOS tend to have a lower average body mass index (BMI) and milder signs of androgen excess than their Western counterparts[13]. It is unclear how East Asian women with PCOS, particularly those who are not obese, would fare in terms of long-term CVD risk. However, this meta-analysis aimed to assess the relationship between PCOS and the risks of CVD in women.

## Materials and Methods

This systematic review was registered in the International Prospective Register of Systematic Reviews (PROSPERO) under the identifier CRD42024625702.

### Search Strategy

We followed the guidelines of Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA-P). We extracted eligible studies from PubMed, Scopus, and Google Scholar databases published in English up to August 2024. The searched keywords included "polycystic ovary syndrome," "PCOS," "sclerocystic ovarian degeneration," "Stein-Leventhal syndrome," "cardiovascular diseases," "myocardial infarction," "coronary heart disease," "cardiovascular stroke," "myocardial infarct," "heart attack," "ischemic heart disease," "myocardial ischemia," "stroke," "cerebrovascular accident," and "apoplexy." Our search strategy is summarized in Table 1.

### Inclusion and Exclusion Criteria

Observational studies investigating the association between PCOS and cardiovascular and cerebrovascular diseases were included. Editorials, conference abstracts, reviews, commentaries, and interventional studies were excluded. Animal studies, as well as non-English articles, were also excluded. Cardiovascular outcomes assessed encompassed both clinical and subclinical disease measures, including incidence, prevalence, and mortality. Cardiovascular-related deaths were defined as those caused by sudden cardiac arrest, acute myocardial infarction, advanced heart failure, peripheral arterial disease, or stroke (Table 2).

### Data Collection and Quality Assessment

Two independent reviewers extracted the data using a consistent and standardized approach. Any disagreements were settled through discussion with a third reviewer. Extracted data included information such as the first author's name, year of publication, study location, average participant age, sample size, follow-up period, type of study, diagnostic criteria for PCOS, and any confounding variables adjusted for.

**Table 1.** Search strategy of PubMed and Scopus databases

| Database | Search strategy | Search date | Results |
|---|---|---|---|
| Scopus | ( TITLE-ABS-KEY ( polycystic AND ovary AND syndrome ) OR TITLE-ABS-KEY ( pcos ) OR TITLE-ABS-KEY ( stein-leventhal AND syndrome ) OR TITLE-ABS-KEY ( sclerocystic AND ovarian AND degeneration ) ) AND ( TITLE-ABS-KEY ( mortality ) OR TITLE-ABS-KEY ( cardiovascular AND death ) OR TITLE-ABS-KEY ( cardiovascular AND diseases ) OR TITLE-ABS-KEY ( cvd ) OR TITLE-ABS-KEY ( coronary AND heart AND disease ) OR TITLE-ABS-KEY ( myocardial AND infarction ) OR TITLE-ABS-KEY ( myocardial AND infarct ) OR TITLE-ABS-KEY ( cardiovascular AND stroke ) OR TITLE-ABS-KEY ( heart AND attack ) OR TITLE-ABS-KEY ( myocardial AND ischemia ) OR TITLE-ABS-KEY ( stroke ) OR TITLE-ABS-KEY ( cerebrovascular ) OR TITLE-ABS-KEY ( apoplexy ) ) AND ( LIMIT-TO ( LANGUAGE , "English" ) ) | 8 August | 3908 |
| Pubmed | ("cerebrovascular"[Title/Abstracts] OR ("cardiovascular system"[MeSH Terms] OR ("cardiovascular"[Title/Abstracts] AND "system"[Title/Abstracts]) OR "cardiovascular system"[Title/Abstracts] OR "cardiovascular"[Title/Abstracts] OR "cardiovasculars"[Title/Abstracts])) AND ("polycystic ovary syndrome"[MeSH Terms] OR ("polycystic"[Title/Abstracts] AND "ovary"[Title/Abstracts] AND "syndrome"[Title/Abstracts]) OR "polycystic ovary syndrome"[Title/Abstracts]) | 8 August | 1850 |

We also recorded outcomes such as cardiovascular and all-cause mortality, overall cardiovascular disease, ischemic heart disease, myocardial infarction, and stroke. The methodological quality of included studies was evaluated using the Joanna Briggs Institute critical appraisal checklist (available at https://jbi.global/critical-appraisal-tools), as summarized in Table 3.

### Data Analysis

Hazard ratios (HR) with 95% confidence intervals (CI) were used to assess mortality risk, while odds ratios (OR) with 95% CI were applied to cardiovascular and cerebrovascular event rates, respectively. Heterogeneity across studies was evaluated using the $I^2$ statistic derived from chi-squared tests. Publication bias was visually assessed using funnel plots. All statistical analyses were conducted using STATA software, version 14.

## Results

### Study Selection

A total of 5,708 studies were found through PubMed and Scopus. After removing 3,433 duplicates, an additional 2,224 were excluded based on their titles and abstracts for not meeting the criteria. This left 51 studies for full-text review, and finally, 19 cohort studies with 1,222,912 participants were included (Figure 1).

### Included Studies

Table 2 summarizes the characteristics of the included studies. The majority were conducted in in the United Kingdom (n=4)[7,14,15], with additional studies from Sweden (n=3)[16-18], USA (n=3)[19-21], Denmark (n=2)[22,23], and single studies from Australia[24], Norway[25], the Netherlands[26], Taiwan[27], Iran[28], Finland[29], and Korea[30]. Most studies followed a cohort design, including 12 prospective and 7 retrospective cohort studies. Assessment of study quality is detailed in Table 2. PCOS diagnosis varied, with the Rotterdam criteria (n=6)[9,17-19,22,28] and International Classification of Diseases (ICD) codes (n=6)[7,22-24,27,29] being the most frequently used methods. Other diagnostic approaches included the NIH criteria (n=2)[9,21], histopathological evaluation, laparoscopic criteria, self-reported characteristics, and androgen excess (n=2)[20,26].

Follow-up durations ranged from 3.83 years to 32 years, and the mean or median age at follow-up spanned from 25 to 81 years. Outcomes assessed included myocardial infarction (MI), cerebrovascular events (stroke or transient ischemic attack), composite CVD outcomes, ischemic heart disease, large-vessel disease, and major adverse cardiovascular events. Data collection methods included questionnaires, medical records, health insurance databases, clinical examinations, and registry records. Most studies adjusted for factors such as age, BMI, and metabolic conditions like diabetes and hypertension.

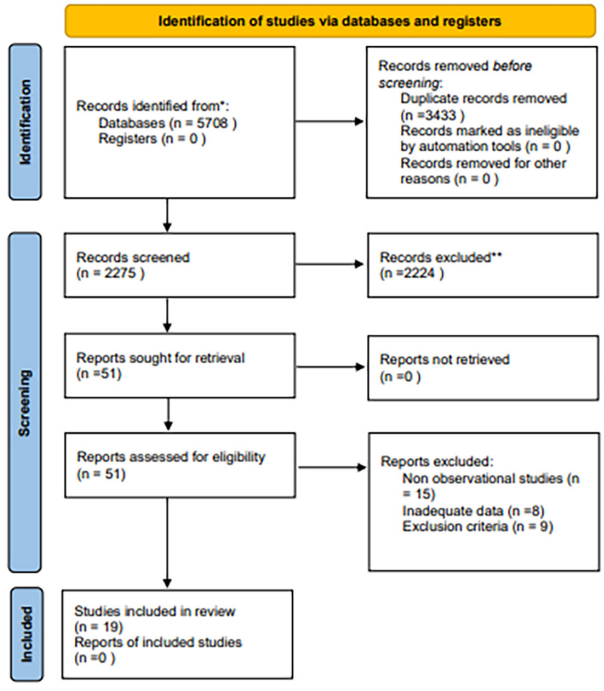


**Figure 1.** Preferred reporting items for systematic review and meta-analysis flow diagram for current systematic review

**Table 2. Overview of included studies**

| Study | Study type | Population source (country) | Sample size (PCOS/control) | Follow-up duration | Mean age at follow-up | Exposure (definition) | Outcome (definition) | Method of data collection | Covariates controlled for |
|---|---|---|---|---|---|---|---|---|---|
| Dahlgren et al.[16] 1992 | Prospective cohort | Sweden | 33/132 | 12 y | PCOS; 45.9 (40-61), age matched controls | Histopathological characteristics | Myocardial infarction | Questionnaire, venous blood sampling | NR |
| Wild et al.[14] 2000 | Prospective cohort | UK | 319/106 | 31 (15-47) y since diagnosis of PCOS | 56.7 (38-98) y, control; 56.7 (38-98) y | Laparoscopic criteria | Cardiovascular endpoints were grouped as composite CVD events, encompassing MI, angina, revascularization procedures, and abnormal treadmill test findings. Cerebrovascular disease outcomes included stroke and TIA) | ICD, patient-reported | Controlled for BMI |
| Lunde and Tanbo[25] 2007 | Prospective cohort | Norway | 131/723 | 15-20 y | NR | Ultrasound examination, with histological examination and two or more of the symptoms | Myocardial infarction, stroke | Patient-reported and checked with the reports from the hospitals | NR |
| Schmidt et al.[18] 2011 | Prospective cohort | Sweden | 25/68 | 21 y | PCOS; 70.4±5.0 y, control; 70.7±5.6 y | Rotterdam | Stroke, MI, cardiovascular disease (including MI and stroke), mortality due to MI | ICD | NR |
| Iftikhar et al.[19] 2012 | Retrospective cohort | USA | 309/343 | 23.7±13.7 | PCOS; 46.7 | Rotterdam | MI, angina, stroke, CABG, composite CVD (MI, angina, stroke, CABG), CVD deaths | Patient-reported or official mortality records | Controlled for age at final assessment, BMI, use of fertility treatments, prior diagnosis of hypertension, postmenopausal hormone therapy exposure, and relevant familial medical history |

**Table 2. Continued**

| Study | Study type | Population source (country) | Sample size (PCOS/control) | Follow-up duration | Mean age at follow-up | Exposure (definition) | Outcome (definition) | Method of data collection | Covariates controlled for |
|---|---|---|---|---|---|---|---|---|---|
| Morgan et al.[15] 2012 | Retrospective cohort | UK | 21,734/ 86,936 | PCOS; 4.7y (median) Controls; 5.8y (median) | 27.1±7.1 both PCOS and control | Medical records | Large-vessel pathology: initial occurrence of myocardial infarction, stroke, angina, or any form of central or peripheral revascularization | Medical records | Controlled for age frequency of general practitioner visits, BMI, and the year of condition diagnosis. |
| Mani et al.[8] 2013 | Prospective cohort | UK | 2301/ local and national population | 5.2±5.1 | 36.3±10.0 | AEPCOS | Cerebrovascular accident cardiovascular death, MI, angina, heart failure, composite CVD outcome | Hospital records | Controlled for BMI, age, hypertension, and diabetes |
| Calderon-Margalit et al.[20] 2014 | Prospective cohort | USA | 55/668 | 20 y | 45.4±3.44; 45.4±3.57 | Oligomenorrhea (self-report), hyperandrogenism (self-reported hirsutism, 95th percentile testosterone levels) | Ischemic heart disease (self-report) | Questionnaire, stored serum samples | controlled for age, racial background, level of formal education, tobacco use, menopausal stage, BMI, systolic blood pressure, log-transformed triglyceride levels, and insulin resistance as measured by HOMA-IR |
| Glintborg et al.[22] 2015 | Prospective cohort | Denmark | 19,199/57,483 | 17 y | OUH: PCOS; 29.3±8.5, Denmark: PCOS; 30.6±9.6, control; 30.6±9.6 | Rotterdam Criteria/ ICD-10 | Cardiovascular disease, Myocardial infarction, Stroke | Medical history, clinical examination, transvaginal ultrasound, and fasting blood samples | NR |
| Hart and Doherty[24] 2015 | Retrospective cohort | Australia | 2566/25,660 | 22 y | 35.8 years (range: 16.6-47.0 y) for both groups | ICD-10 or ICD-9 | Cerebrovascular conditions, ischemic heart disease. | ICD-10 | Controlled for age, BMI |
| Merz et al.[21] 2016 | Prospective cohort | USA | 25/27 | 10 y | 62.6±11.6, control; 64.8±9.6 | NIH | CAD, Composite CVD (including MI, stroke, and cardiovascular death), CVD death (including sudden cardiac deaths, CHF, MI, PAD, and stroke) | Angiogram results, official mortality records, family-provided data, and clinical documentation | Controlled for DM, waist circumference, hypertension, and angiographic CAD |

Table 2. Continued

| Study | Study type | Population source (country) | Sample size (PCOS/control) | Follow-up duration | Mean age at follow-up | Exposure (definition) | Outcome (definition) | Method of data collection | Covariates controlled for |
|---|---|---|---|---|---|---|---|---|---|
| Meun et al.[26] 2018 | Prospective cohort | Netherlands | 106/171 | 11.36 y (median) | 69.57±8.72, control; 69.20±8.60 | High FAI (highest quartile vs middle two) | PAD, CHD, stroke, CVD (medical notes) | Interview, examinations, medical notes | Controlled for age, WHR, time passed since menopause, cohort classification, lipid profile (total and HDL cholesterol), smoking behavior, systolic blood pressure, use of antihypertensive therapy, diabetes status, and hormone therapy usage. |
| Ding et al.[27] 2018 | Retrospective cohort | Taiwan | 8,048/32,192 | 5.9 y (median) | 28.11 y | ICD-9 | Coronary artery disease (ICD-9-CM) | Health insurance database | Controlled for age, obesity status, history of DM, hypertension, lipid disorders, atrial fibrillation, chronic renal impairment, and evidence of arterial plaque formation. |
| Oliver-Williams et al.[23] 2021 | Retrospective cohort | Denmark | 6,149/54,426 | 8.9 y | 32.9 (29.7-36.5) | ICD-10 | Cardiovascular disease (ICD-10), death | Registry records | Controlled for age, timing of initial ART intervention, parity at study entry, presence of gestational diabetes, marital or relationship status, and educational background. |
| Berni et al.[7] 2021 | Retrospective cohort study | UK | 174,660/174,660 | PCOS; 3.83 (1.89-7.78)/ control; 3.00 (1.37-6.36) | 29 (24.00-34.00) | ICD-10 | Time to significant adverse cardiovascular event (MACE), comprising MI, stroke, angina, revascularization procedures, and cardiovascular mortality. | Patient electronic healthcare records (EHR) recovered regularly in primary care | Controlled for age, BMI classification, tobacco and alcohol use, presence of T2DM, overall baseline comorbidity burden as quantified by the Charlson Comorbidity Index, systolic and diastolic blood pressure, and socioeconomic status using the Index of Multiple Deprivation (IMD) quintiles) |

**Table 2. Continued**

| Study | Study type | Population source (country) | Sample size (PCOS/control) | Follow-up duration | Mean age at follow-up | Exposure (definition) | Outcome (definition) | Method of data collection | Covariates controlled for |
|---|---|---|---|---|---|---|---|---|---|
| Forslund et al.[17] 2022 | Prospective cohort with cross-sectional analysis | Sweden | 35/99 | 32 y | 81 y | Rotterdam criteria | All-cause mortality, CVD-related mortality, all CVD, Myocardial infarction, Stroke/TIA | Patients' medical records, registry records | NR |
| Mahboobifard et al.[28] 2022 | Prospective cohort with longitudinal analysis | Iran | 356/1235 | 15.4 y | 29.7±6.8 (PCOS) 31.1±7.6 (control) | Rotterdam criteria | Prevalence and incidence: CVD (including stroke, MI, angina, angiographic evidence), silent CVD (indicated by potential and probable ECG changes) | Patient-reported and confirmed by medical interview and documents | Controlled for age, BMI, smoking habit, hypertension, DM, and lipid profile |
| Ollila et al.[9] 2023 | Prospective, population-based cohort study | Northern Finland | NIH-PCOS (144)/Non-NIH (2,051) Rotterdam-PCOS (386)/ non-Rotterdam (1518) | 22 y | From 31 to 53 | National Institute of Health (NIH) criteria (n=144) or the Rotterdam criteria (n=386) | Major adverse cardiovascular events (MACE), including myocardial infarction (MI), stroke, heart failure and cardiovascular mortality | Comprehensive questionnaires and clinical examinations | Controlled for BMI |
| Ryu et al.[29] 2024 | Retrospective matched cohort study | Korea | 137,416/412,118 | 4.0 y (PCOS) 4.5 y (Control) | 30.4±5.5 | ICD-10 | Ischemic heart disease, cerebrovascular diseases, combined cardiocerebrovascular diseases | Health insurance claims | Controlled for age, BMI, prior diagnoses of diabetes, hypertension, and lipid disorders, as well as lifestyle factors including physical activity, alcohol intake, and smoking status. Blood pressure (systolic/diastolic), total cholesterol, and triglyceride concentrations were also included |

Mean (range), Mean ± SD

PCOS: Polycystic ovary syndrome, CVD: Cardiovascular disease, MI: Myocardial infarction, TIA: Transient ischemic attack, MACE: Major adverse cardiovascular event, CAD: Coronary artery disease, CABG: Coronary Artery bypass grafting, FAI: Free androgen index, ICD: International classification of diseases, SBP: Systolic blood pressure, TG: Triglycerides, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, WHR: Waist-to-hip ratio, NR: Not reported, BMI: Body mass index, DM: Diabetes mellitus, CHF: Congestive heart failure, OUH: Odense University Hospital, SD: Standard deviation

**Table 3.** Quality assessment of included studies

| Study | Were the two groups similar and recruited from the same population? | Were the exposures measured similarly to assign people to both exposed and unexposed groups? | Was the exposure measured in a valid and reliable way? | Were confounding factors identified? | Were strategies to deal with confounding factors stated? | Were the groups/ participants free of the outcome at the start of the study (or at the moment of exposure)? | Were the outcomes measured in a valid and reliable way? | Was the follow up time reported and sufficient to be long enough for outcomes to occur? | Was follow up complete, and if not, were the reasons to loss to follow up described and explored? | Were strategies to address incomplete follow up utilized? | Was appropriate statistical analysis used? |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Dahlgren et al.[16] 1992 | ✓ | ✓ | ✓ | × | × | ✓ | × | ✓ | ✓ | NA | ✓ |
| Wild et al.[14] 2000 | ✓ | ✓ | ✓ | Unclear | × | ✓ | ✓ | ✓ | ✓ | NA | ✓ |
| Lunde and Tanbo[25] 2007 | ✓ | ✓ | ✓ | × | × | ✓ | ✓ | ✓ | ✓ | NA | ✓ |
| Schmidt et al.[18] 2011 | ✓ | ✓ | ✓ | × | × | ✓ | ✓ | ✓ | ✓ | NA | ✓ |
| Iftikhar et al.[19] 2012 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | NA | ✓ |
| Morgan et al.[15] 2012 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | NA | ✓ |
| Mani et al.[8] 2013 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | NA | ✓ |
| Calderon-Margalit et al.[20] 2014 | ✓ | ✓ | × | ✓ | ✓ | ✓ | × | ✓ | ✓ | NA | ✓ |
| Glintborg et al.[23] 2015 | ✓ | ✓ | ✓ | × | × | ✓ | ✓ | ✓ | ✓ | NA | ✓ |
| Hart and Doherty[24] 2015 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | NA | ✓ |
| Merz et al.[21] 2016 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | NA | ✓ |
| Meun et al.[26] 2018 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | NA | ✓ |
| Ding et al.[27] 2018 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | NA | ✓ |

**Table 3.** Continued

| Study | Were the two groups similar and recruited from the same population? | Were the exposures measured similarly to assign people to both exposed and unexposed groups? | Was the exposure measured in a valid and reliable way? | Were confounding factors identified? | Were strategies to deal with confounding factors stated? | Were the groups/ participants free of the outcome at the start of the study (or at the moment of exposure)? | Were the outcomes measured in a valid and reliable way? | Was the follow up time reported and sufficient to be long enough for outcomes to occur? | Was follow up complete, and if not, were the reasons to loss to follow up described and explored? | Were strategies to address incomplete follow up utilized? | Was appropriate statistical analysis used? |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Oliver-Williams et al.[23] 2021 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | NA | ✓ |
| Berni et al.[7] 2021 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | NA | ✓ |
| Forslund et al.[17] 2022 | ✓ | ✓ | ✓ | × | × | ✓ | ✓ | ✓ | ✓ | NA | ✓ |
| Mahboobifard et al.[28] 2022 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | NA | ✓ |
| Ollila et al.[9] 2023 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | NA | ✓ |
| Ryu et al.[29] 2024 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | NA | ✓ |

### All-Cause Mortality

Seven studies evaluated the risk of all-cause mortality in PCOS versus non-PCOS groups. No significant difference in risk was observed between the two groups (HR: 0.98, 95% CI: 0.80-1.15, $I^2$=0%) in a random-effects model (Figure 2).

### Cardiovascular Death

Five studies assessed the risk of cardiovascular death. Similarly, there was no significant change in risk for the PCOS group compared to the non-PCOS group (HR: 1.75, 95% CI: -2.20-5.71, $I^2$=38.4%) in a random-effects model (Figure 3).

### Any CVD

Nineteen studies evaluated the risk of any CVD. Patients with PCOS did not have a significantly higher risk compared to those without PCOS (HR: 1.80, 95% CI: -5.43-9.04, $I^2$=0%) in a random-effects model (Figure 4).

### Myocardial Infarction

Twelve studies investigated the risk of MI. There was no significant difference between the PCOS and non-PCOS groups (HR: 2.68, 95% CI: 0.69-4.82, p=0.003; $I^2$=82%, p<0.00001) in a random-effects model (Figure 5).

### Ischemic Heart Disease

Seven studies evaluated ischemic heart disease outcomes. The analysis showed no significant increase in risk for patients with PCOS compared to controls (HR: 2.68, 95% CI: 0.69-4.67, $I^2$=99.8%) in a random-effects model, Figure 6).

### Stroke

Eleven studies assessed the risk of stroke. Unlike other outcomes, PCOS was associated with a significantly increased risk of stroke (OR: 1.89, 95% CI: 1.22-2.55, $I^2$=97.7%) in a random-effects model (Figure 7).

### Publication Bias

Publication bias for CVD death risk was assessed using Egger's regression test, Begg's test, and funnel plot analysis. While Begg's test indicated no bias (p=1.00), Egger's regression test and the funnel plot (Figure 8) revealed evidence of publication bias (p=0.01).

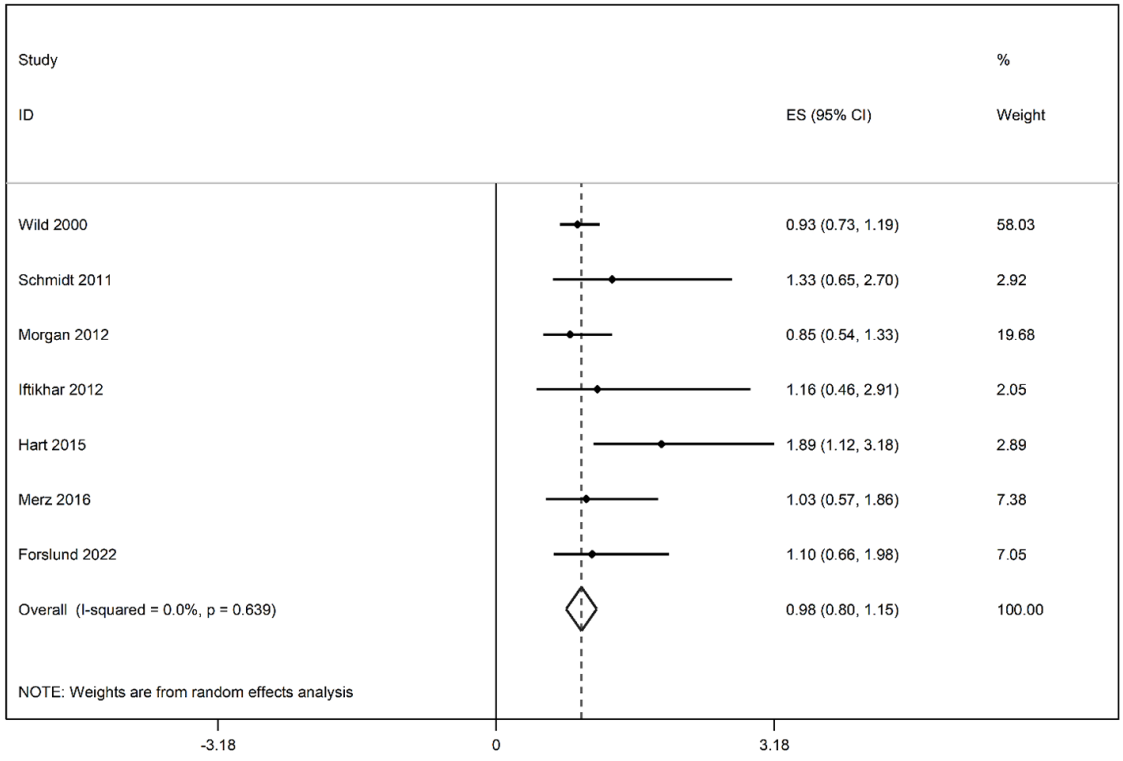


**Figure 2.** Forest plot for all-cause mortality

*CI: Confidence interval*

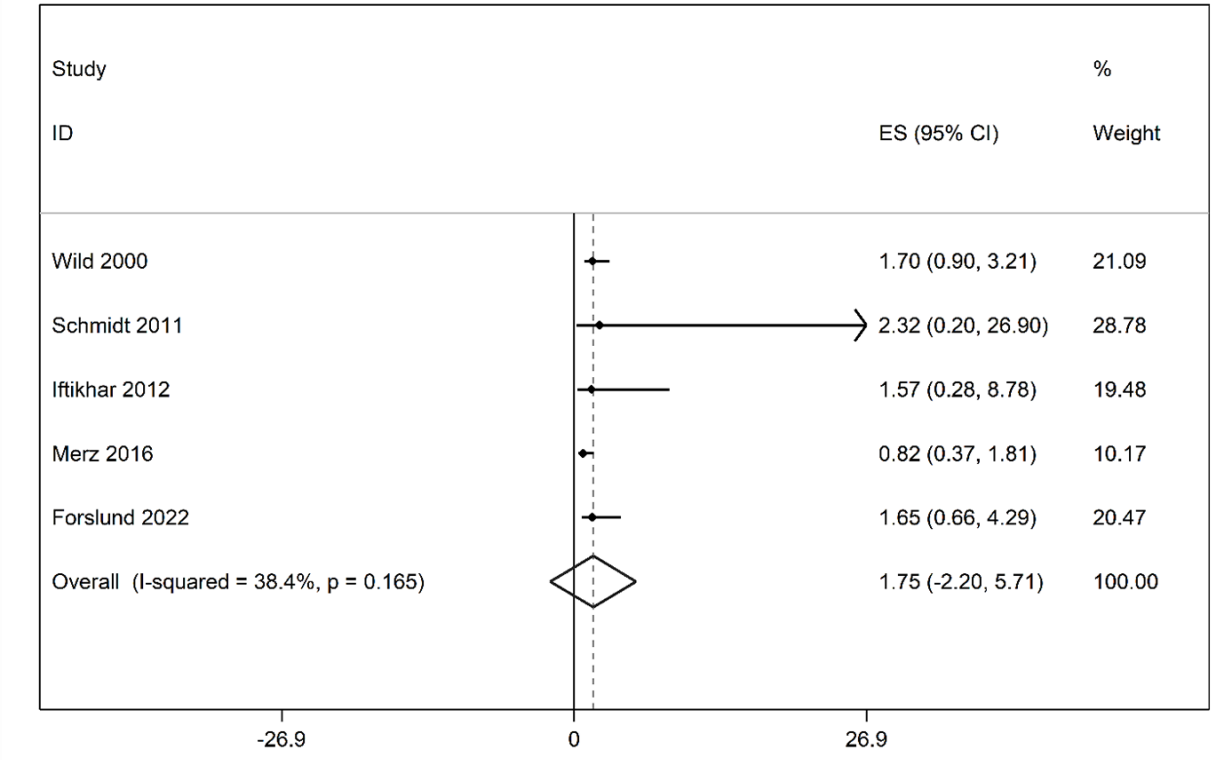


**Figure 3.** Forest plot for CVD death

*CVD: Cardiovascular disease, CI: Confidence interval*

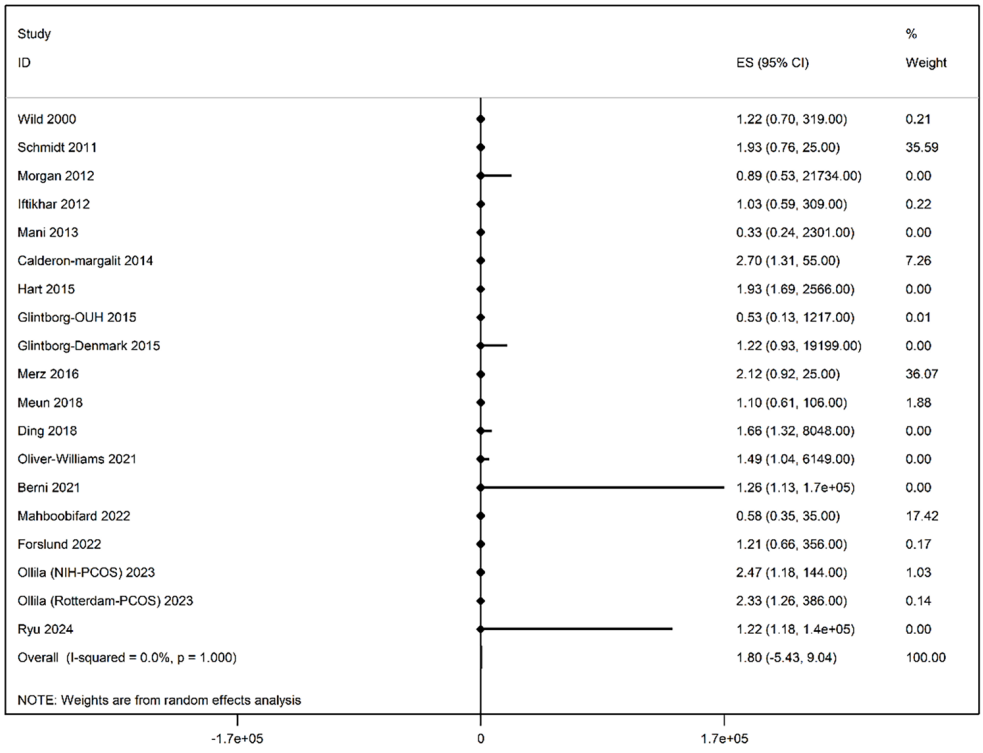


**Figure 4.** Forest plot for any CVD

*CVD: Cardiovascular disease, CI: Confidence interval*

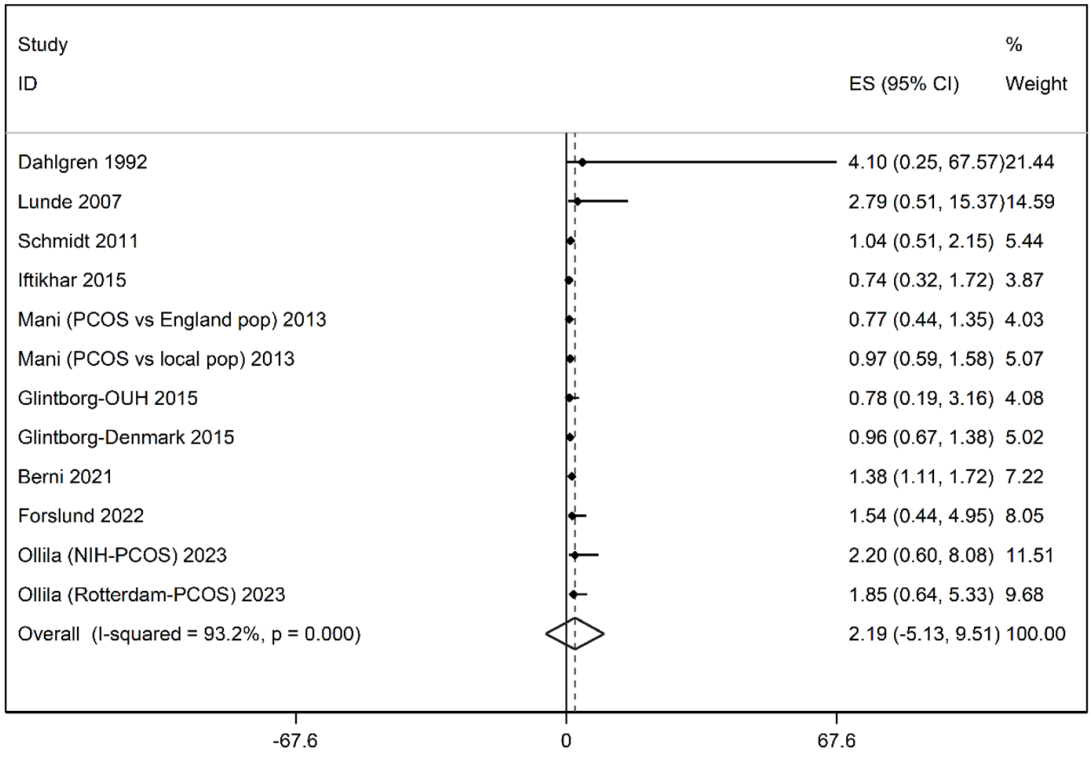


**Figure 5.** Forest plot for MI

*MI: Myocardial infarction, CI: Confidence interval*

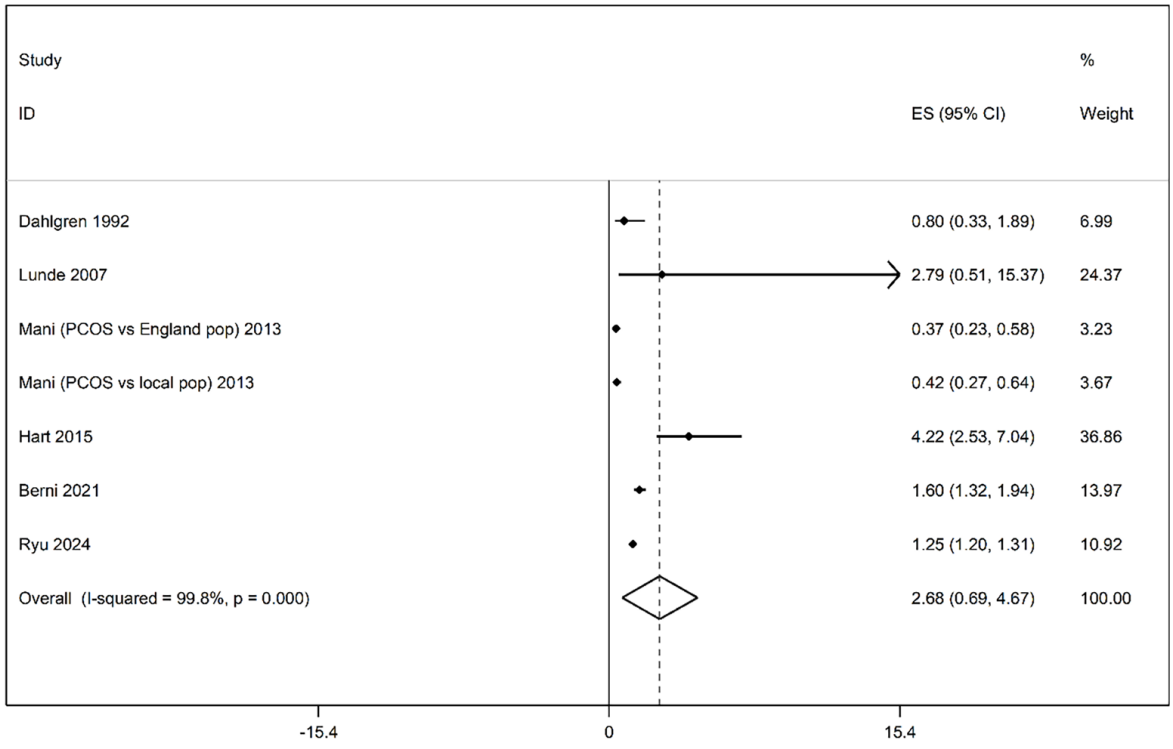


**Figure 6.** Forest plot for IHD

*IHD: Ischemic heart disease, CI: Confidence interval*

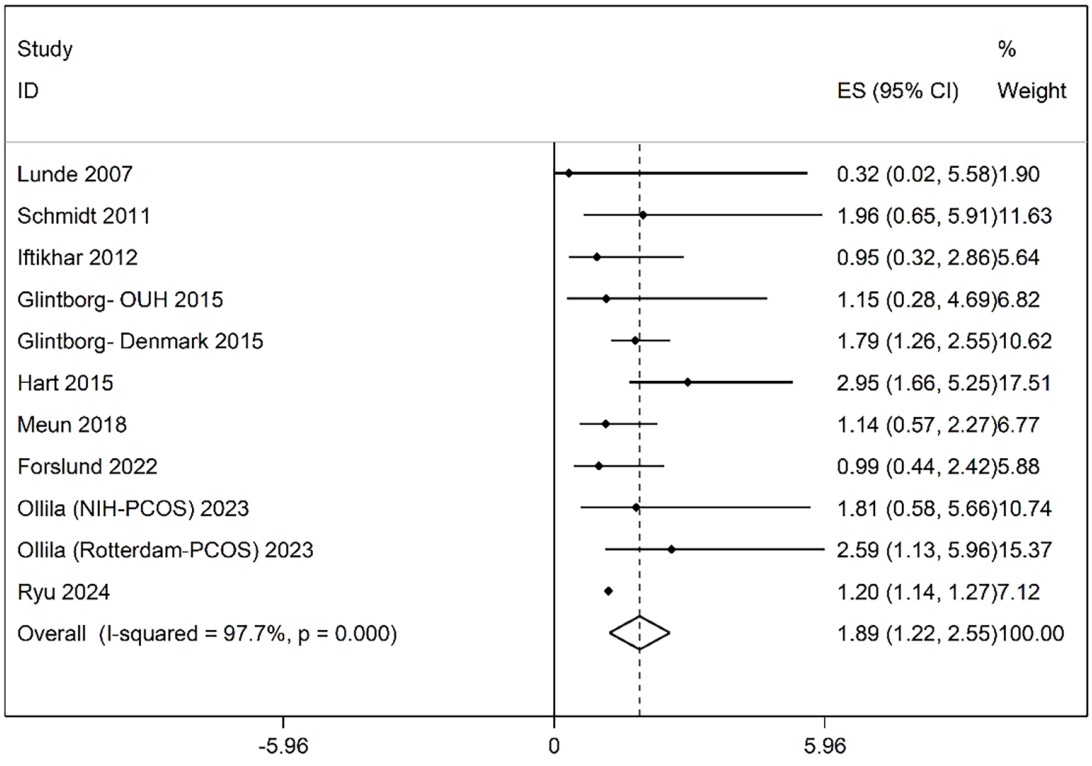


**Figure 7.** Forest plot for stroke

*CI: Confidence interval*

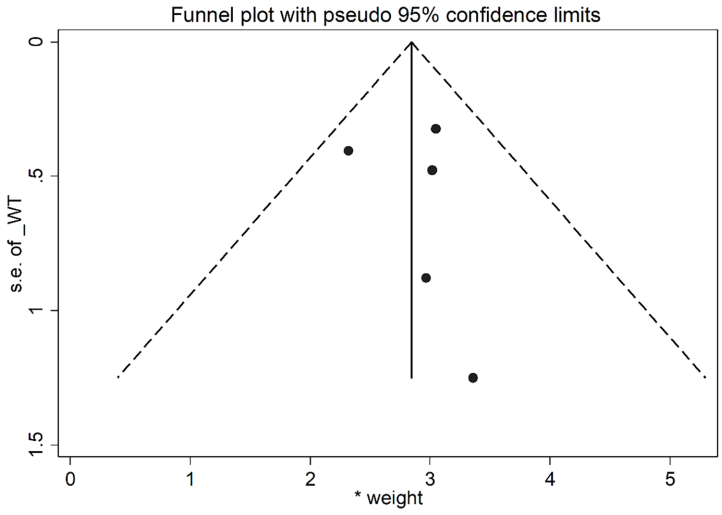


**Figure 8.** Funnel plot for IHD
*IHD: Ischemic heart disease*

## Discussion

This meta-analysis investigated the relationship between PCOS and cardiovascular outcomes, including all-cause mortality, cardiovascular mortality, MI, IHD, stroke, and overall CVD. Our findings indicate that while women with PCOS are at an increased risk for stroke, no significant association was observed for other cardiovascular outcomes, underscoring the need for further investigation into the specific pathways underlying these risks.

The current body of evidence on the impact of PCOS on CVD risk remains inconsistent. Although multiple studies have linked PCOS with various cardiometabolic abnormalities such as diabetes[30], dyslipidemia[31,32], hypertension[31], and metabolic syndrome[30], the direct connection to clinical cardiovascular events is not yet clear. IR and hyperinsulinemia, both of which are common in PCOS, contribute to oxidative stress, vascular dysfunction, and reduced vascular compliance, all of which increase the risk of CVD[33]. Furthermore, PCOS leads to dysregulation of lipid metabolism, resulting in elevated levels of low-density lipoprotein and triglycerides and reduced high-density lipoprotein, which exacerbate the risk of atherosclerosis and dyslipidemia[34]. The presence of excess adipose tissue in women with PCOS also raises levels of inflammatory cytokines and leptin, further worsening IR and promoting hypertension[35]. Previous systematic reviews have presented mixed findings regarding the association between PCOS and cardiovascular outcomes. For instance, De Groot et al.[36] reported that women with PCOS had approximately twice the risk of developing coronary heart disease and experiencing strokes. A review by Millán-de-Meer et al.[32] showed the prevalence of cardiovascular outcomes in both premenopausal and postmenopausal women, displaying a notable increase in OR for MI and stroke, though no remarkable increase was observed for overall CVD or coronary artery disease. A 2020 meta-analysis by Ramezani Tehrani et al.[37] found that reproductive-aged women with PCOS had a significantly higher HR for clinical cardiovascular events. More recently, studies by Tay et al.[38] and Zhang et al.[39] have indicated an elevated risk of myocardial infarction, ischemic heart disease, and stroke in women with polycystic ovary syndrome. Despite these findings, neither study reported a significant association between PCOS and either all-cause or cardiovascular-specific mortality, highlighting the complexity of this relationship.

Several methodological limitations must be considered when interpreting these findings. Many of the studies included in this analysis had small sample sizes, short follow-up periods, and primarily focused on younger women, which may limit the generalizability of the results. Additionally, there was inconsistency in the diagnostic criteria for PCOS across studies, and the inclusion of different PCOS phenotypes introduced heterogeneity. Importantly, the cardiovascular impact of different PCOS phenotypes is not uniform. Women with oligo-amenorrhea or menstrual irregularities appear to be at a higher risk for CVD, likely due to the effects of hyperinsulinemia and IR. On the other hand, the evidence linking hyperandrogenism to cardiovascular outcomes remains mixed[40].

Age also appears to be an important factor influencing cardiovascular risk in women with PCOS[37]. Younger women tend to have higher cardiovascular risk due to factors such as central obesity, IR, and unfavorable lipid profiles[41,42]. However, these risks often decrease with age[43], as androgen excess and metabolic abnormalities tend to improve over time[44,45]. The wide age range of participants in many cohort studies could have obscured significant associations between PCOS and cardiovascular outcomes, especially among older women.

Geographic and socioeconomic disparities should also be considered when interpreting these findings. Recent evidence suggests that cardiovascular risks associated with PCOS may be more pronounced in East Asian and African populations, particularly in lower-income countries[46]. Variations in healthcare access, lifestyle, and socioeconomic conditions likely contribute to these differences[14].

Despite the high prevalence of metabolic abnormalities in women with PCOS, the risk of cardiovascular events is not uniformly elevated across all individuals. Several protective factors may contribute to a more favorable cardiovascular profile in some women with PCOS. For example, women with PCOS tend to experience delayed menopause, and earlier menarche, which may lead to an extended exposure to the cardio-protective effects of estrogen. Additionally, due to heightened awareness of PCOS, proactive management of cardiovascular risk factors may mitigate some of the cardiovascular risks[47,48].

Although the exact impact of PCOS on cardiovascular health remains unclear, existing guidelines recommend preventive measures due to the high prevalence of cardiometabolic issues among affected individuals. These guidelines suggest that weight should be tracked in a non-stigmatizing and supportive manner, and lipid profiles should be first assessed at diagnosis, and thereafter, periodically based on overall cardiovascular risk. BP should be measured once a year, and an oral glucose tolerance test should be conducted at the time of diagnosis, with follow-up tests up to three times per year or more frequently if there are elevated diabetes risk factors or if pregnancy is being planned or achieved[49,50].

In conclusion, while PCOS is associated with significant metabolic and vascular abnormalities, the clinical translation to cardiovascular events is complex and influenced by factors such as age, phenotype, geography, and proactive risk management. Future research should focus on phenotype-specific risks, long-term outcomes, and diverse populations to better clarify the cardiovascular implications of PCOS.

### Strengths and Limitations

This meta-analysis has several strengths. It includes a large sample size of over one million women and examines a broad range of cardiovascular outcomes, providing a comprehensive view of the relationship between PCOS and cardiovascular health. The inclusion of longitudinal studies, many of which had follow-up periods of 10 years or more, adds reliability to the findings. However, several limitations need to be acknowledged. Despite searching multiple databases and reviewing reference lists of prior studies, some relevant studies may have been missed. There was significant variability in how cardiovascular outcomes and study designs were defined across the included studies. Many studies relied on ICD codes to classify cardiovascular events. While widely used, these codes can be inaccurate. Additionally, some studies used questionnaires or self-reported data to collect information, which may introduce bias. Another challenge was the inconsistency in diagnostic criteria for PCOS. Different studies used varying definitions, such as the Rotterdam criteria, and others, which capture a range of PCOS phenotypes. This lack of standardization makes it difficult to differentiate cardiovascular risks associated with different PCOS subtypes. Moreover, most studies focused on premenopausal women, providing limited insights into how cardiovascular risks may change in aging women with PCOS. Therefore, further research is needed to address these limitations and provide more definitive answers regarding the long-term cardiovascular risks associated with PCOS.

### Implications for Practice

This meta-analysis highlights the need for proactive management of cardiovascular risks in women with PCOS, particularly given the increased risk of stroke. Regular monitoring of blood pressure, glucose, and lipid levels, along with lifestyle modification, should be promoted. Care should be personalized based on age and PCOS phenotype, with younger women requiring a focus on metabolic health and older women on long-term vascular risk reduction. Addressing geographic and socioeconomic disparities is also essential to improve access to preventive care. Educating patients about their risks and promoting healthy lifestyles can further reduce complications.

## Conclusion

This meta-analysis demonstrates an elevated risk of stroke among women with PCOS, but the evidence linking PCOS to other cardiovascular outcomes, for instance, MI and overall cardiovascular mortality, remains unclear. Although PCOS is frequently linked to metabolic disturbances like IR, abnormal lipid profiles, and high blood pressure, the impact of these factors on clinical cardiovascular events is complex and influenced by age, phenotype, geography, and preventive management. Future research should focus on phenotype-specific risks and larger, long-term studies to better understand the cardiovascular implications of PCOS.

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### Footnotes

### Authorship Contributions

Concept: S.T.M., P.B.A., Design: S.T.M., P.B.A., Data Collection or Processing: A.A., H.P., R.K., Analysis or Interpretation: S.T.M., P.B.A., Literature Search: A.A., H.P., R.K., Y.J., Writing: S.T.M., A.A., R.K., Y.J.

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