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TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

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Turkish Journal of Obstetrics and Gynecology (formerly called Türk Jinekoloji ve Obstetrik Derneği Dergisi) is the official peer-reviewed journal of the Turkish Society of Obstetrics and Gynecology and is published quarterly on March, June, September and December.

It is an independent peer-reviewed international journal published in English language since 2014 September. Manuscripts are reviewed in accordance with "double-blind peer review" process for both referees and authors.

The target audience of Turkish Journal of Obstetrics and Gynecology includes gynecologists, obstetricians, urogynecologists, reproductive medicine specialists, gynecological oncologists and primary care physicians interested in gynecology practice. It publishes original work on all aspects of obstetrics and gynecology. The aim of Turkish Journal of Obstetrics and Gynecology is to publish high quality original research articles. In addition to research articles, reviews, editorials, letters to the editor and case presentations are also published.

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Instructions for authors page of the journal is available in the journal content and at www.tjoddergisi.org

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The "Turkish Journal of Obstetrics and Gynecology" is the official publication of the Turkish Society of Obstetricians and Gynecologists. The journal is published quarterly (March, June, September and December) in English and publishes original peer-reviewed articles, reviews, case reports and commentaries in the fields of gynecology, gynecologic oncology, endocrinology and reproductive medicine and obstetrics. The journal gives publication priority to original research articles over case reports. Reviews are considered for publication only if they are prepared by authors who have at least three published manuscripts in international peer-reviewed journals on the topic of the review and these studies should be cited in the review. Otherwise only invited reviews will be considered for peer-review from qualified experts in the area.

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appropriately investigated and resolved. The statement about the authors' contributions should be placed in the cover letter. All persons who contributed to the work, but not sufficiently to be authors, must be acknowledged.

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Cover letter to the editors addressing the following points:

· The authors' intent to submit solely to Turkish Journal of Obstetrics and Gynecology.

· Verification that the manuscript is not under consideration elsewhere, and indication from the authors that it will not be submitted elsewhere until a final decision is made by the editors of Turkish Journal of Obstetrics and Gynecology.

· The declaration of transparency from the corresponding author.

· Clinical trial registration, if applicable.

· Institutional review board (IRB) approval or exemption.

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Preparation of Manuscripts

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CONSORT statement for randomized controlled trials (Moher D, Schultz KF, Altman D, for the CONSORT Group. The CONSORT statement revised recommendations for improving the quality of reports of parallel group randomized trials. JAMA 2001; 285: 1987-91) (http://www.consort-statement.org/),

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

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

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

MOOSE guidelines for meta-analysis and systemic reviews of observational studies (Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting Meta-analysis of observational Studies in Epidemiology (MOOSE) group. JAMA 2000; 283: 2008-12).

CARE guidelines are designed to increase the accuracy, transparency, and usefulness of case reports. (Gagnier JJ, Kienle G, Altman DG, Moher D, Sox H, Riley D; the CARE Group. The CARE Guidelines: Consensus-based Clinical Case Reporting Guideline Development.) (http://www.care-statement.org/)

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Authors must provide statement on the absence of conflict of interests between authors and provide authorship contributions and declare if any financial/material support.

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All manuscripts must be submitted as Microsoft Word (.doc or .docx) files. All manuscript pages (including references, tables, and figure legends) must be double-spaced. Use a standard, 12-point typeface such as Times New Roman. Top, bottom, and side margins should be set at 1 inch. Authors must include the following in the manuscript file:

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Title Page

A separate title page should list;

-The manuscript title, which should contain no more than a total of 100 characters (counting letters and spaces) and should not be declarative; do not use abbreviations or commercial names in the title.

- A short title of no more than 50 characters, including spaces, for use as a running foot.

- All author name(s), institutional, corporate, or commercial affiliations, and up to two major degree(s).

- Corresponding author's name, address, telephone (including the mobile phone number), fax numbers and e-mail address (the corresponding author will be responsible for all correspondence and other matters relating to the manuscript).

Precis

The precis is a one-sentence synopsis of no more than 30 words that describes the basic findings of the article. Precis sample can be seen below:

'Using a 45 point questionnaire, we have evaluated the trend of Robotic surgery training in the gynecologic surgery fellowship programs across the nation'.

Abstract

All manuscripts should be accompanied by an abstract. All information in the abstract should be consistent with the information in the text, tables, or figures. Avoid use of commercial names in the abstract. Original research reports should have a structured abstract of no more than 250 words, using the following headings:

· Objective: Main question, objective, or hypothesis (single phrase starting with, for example, "To evaluate..." or "To estimate." [never start with "To determine."]).

· Materials and Methods: Study design, participants, outcome measures, and in the case of a negative study, statistical power.

· Results: Measurements expressed in absolute numbers and percentages, and when appropriate indicate relative risks or odds ratios with confidence intervals and level of statistical significance; any results contained in the abstract should also be presented in the body of the manuscript, tables, or figures.

· Conclusion: Directly supported by data, along with clinical implications.

Authors from Turkey or Turkish speaking countries are expected to submit a Turkish abstract including subheadings such as "Amaç, Gereç ve Yöntemler, Bulgular, Sonuç". The abstract of Authors whose native language is not Turkish will be provided free of charge translation services into Turkish language.

A structured abstract is not required with review articles and case reports.

Keywords

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

Turkish abstracts should have keywords "Anahtar Kelimeler" picked from www.atifdizini.com under "Türkiye Bilim Terimleri" link.

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Table 1. Manuscript length at a glance

Article type	Abstract Length	Manuscript Word Count*	Maximum Number of Authors	Maximum Number of References$^{\Phi}$
Original Research	250 words	,500 words (~22 pages)$^{\Psi}$	NA	30
Case report	150 words	,000 words (~8 pages)	4	8
Systematic review	300 words	6,250 words (~25 pages)	4	60
Current commentary	250 words	,000 words (~12 pages)	4	12
Procedure and Instruments	200 words	,000 words (~8 pages)	4	10
Letters	NA	350 words	4	5

*Manuscript length includes all pages in a manuscript (ie, title page, abstract, text, references, tables, boxes, figure legends, and appendixes). $^{\Phi}$Suggested limit. $^{\Psi}$The Introduction should not exceed 250 words. ~approximately; NA, not applicable.

Original researches should have the following sections;

Introduction

State concisely the purpose and rationale for the study and cite only the most pertinent references as background. Avoid a detailed literature review in this section.

Materials and Methods

Describe the research methodology (the patients, experimental animals, material and controls, the methods and procedures utilized, and the statistical method(s) employed) in sufficient detail so that others could duplicate the work. Identify methods of statistical analysis and when appropriate, state the basis (including alpha and beta error estimates) for their selection. Cite any statistical software programs used in the text. Express p values to no more than two decimal places. Indicate your study's power to detect statistical difference.

Address "IRB" issues and participants informed consent as stated above, the complete name of the IRB should be provided in the manuscript. State the generic names of the drugs with the name and country of the manufactures.

Results

Present the detailed findings supported with statistical methods. Figures and tables should supplement, not duplicate the text; presentation of data in either one or the other will suffice. Authors should report

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outcome data as both absolute and relative effects since information presented this way is much more useful for clinicians. Actual numbers and percentages should be given in addition to odds ratios or relative risk. When appropriate, number needed to treat for benefits (NNTb) or harm (NNTh) should be supplied. Emphasize only your important observations; do not compare your observations with those of others. Such comparisons and comments are reserved for the discussion section.

Discussion

Begin with a description of what your study found in relation to the purpose or objectives as stated in the Introduction. State the importance and significance of your findings to clinicians and actual patient care but do not repeat the details given in the Results section. Limit your opinions to those strictly indicated by the facts in your report. Compare your finding with previous studies with explanations in cases where they differ, although a complete review of the literature is not necessary.

Study Limitations

Provide information on the limitations of the study. No new data are to be presented in this section. A final summary is not necessary, as this information should be provided in the abstract and the first paragraph of the Discussion. Although topics that require future research can be mentioned, it is unnecessary to state, "Further research is needed."

Conclusion

The conclusion of the study should be highlighted. The study's new and important findings should be highlighted and interpreted.

Conflict of Interest

Authors must indicate whether or not they have a financial relationship with the organization that sponsored the research.

The main text of case reports should be structured with the following subheadings:

Introduction, Case Report, Discussion and References.

References

References are numbered (Arabic numerals) consecutively in the order in which they appear in the text (note that references should not appear in the abstract) and listed double-spaced at the end of the manuscript. The preferred method for identifying citations in the text is using within parentheses. Use the form of the "Uniform Requirements for Manuscripts" (http://www.icmje.org/about-icmje/faqs/icmje-recommendations/). If number of authors exceeds seven, list first 6 authors followed by et al.

Use references found published in peer-reviewed publications that are generally accessible. Unpublished data, personal communications, statistical programs, papers presented at meetings and symposia, abstracts, letters, and manuscripts submitted for publication cannot be listed in the references. Papers accepted by peer-reviewed publications but not yet published ("in press") are not acceptable as references.

Journal titles should conform to the abbreviations used in "Cumulated Index Medicus".

Examples

Journals; Zeyneloglu HB, Onalan G. Remedies for recurrent implantation failure. Semin Reprod Med 2014;32:297-305.

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Tables should be included in the main document after the reference list. Color figures or gray-scale images must be at minimum 300 DPI resolutions. Figures should be submitted in "*.tiff", "*.jpg" or "*.pdf" format and should not be embedded in the main document. Tables and figures consecutively in the order they are referred to within the main text. Each table must have a title indicating the purpose or content of the table. Do not use internal horizontal and vertical rules. Place explanatory matter in footnotes, not in the heading. Explain all abbreviations used in each table in footnotes. Each figure must have an accompanying descriptive legend defining abbreviations or symbols found in the figure. If photographs of people are used, the subjects must be unidentifiable and the subjects must have provided written permission to use the photograph. There is no charge for color illustrations.

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Units of measurement should be in Systéme International (SI) units. Abbreviations should be avoided in the title. Use only standard abbreviations. If abbreviations are used in the text, they should be defined in the text when first used.

Revisions

Revisions will be sent to the corresponding author. Revisions must be returned as quickly as possible in order not to delay publication. Deadline for the return of revisions is 30 days. The editorial board retains the right to decline manuscripts from review if authors' response delays beyond 30 days. All reviewers' comments should be addressed a revision note containing the author's responses to the reviewers' comments should be submitted with the revised manuscript. An annotated copy of the main document should be submitted with revisions. The Editors have the right to withdraw or retract the paper from the scientific literature in case of proven allegations of misconduct.

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LETTER FROM THE PRESIDENT

Dear Members of the TJOD,

We take great pride in presenting the last issue of the Turkish Journal of Turkish Journal of Obstetrics and Gynecology for the year 2023. This esteemed scientific publication, representing our association, continues to gain recognition in the global scientific community, with its increasing scientific value and growing prestige on both national and international platforms. One noteworthy indicator of our journal's success is the rising number of authors seeking publication on our pages, particularly evident in the increased volume of articles, especially those received from abroad and accepted for publication in our latest issue.

At TJOD, we place significant emphasis on scientific research and knowledge. While our journal stands as a primary testament to this commitment, we also disseminate current scientific findings through the meetings we convene. Recently, we successfully organized the 2nd In Vitro Fertilization and Infertility Congress in Cyprus (TÜBİD) from September 28 to October 1, 2023. The congress featured 35 scientific sessions and 4 keynote lectures, drawing the participation of 168 scientific officers and 30 sponsor companies. With a total of 1,072 participants, we concluded the event on a high note. On behalf of both myself and the Board of Directors, I extend our heartfelt gratitude to everyone who contributed. We are directing our efforts toward the upcoming 21st National Gynecology and Obstetrics Congress.

Despite the great pain experienced by our nation in 2023, I express the hope that 2024 will usher in a year of love, peace, happiness, and tranquility for our country, and I extend my love and respect to all my colleagues and the TJOD family.

Bulent Tiras, Prof. MD
President of TJOD

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EDITORIAL

Dear Colleagues,

We are pleased to present the final issue of 2023. As part of the Turkish Journal of Obstetrics and Gynecology family, it is important to highlight that a substantial number of studies from the international scientific community are consistently submitted to our journal. Upon closer examination of the articles accepted for publication in this issue, it is evident that the majority of them are of international origin.

As is known, our journal embarked on a renewal process some time ago, and as the editorial team, we would like to provide a brief overview of our progress since then. Notably, the readership of our journal has shown a consistent upward trajectory each year. The number of reads, which stood at 116,894 in 2018, 261,275 in 2019, 294,759 in 2020, 314,882 in 2021, and 377,317 in 2022, is anticipated to reach approximately 420,000 in 2023. Furthermore, the number of citations to our journal was 124 in 2018, 196 in 2019, 314 in 2020, 428 in 2021, and 459 in 2022, which has steadily increased. Concerning the h-index values, we also observe a rise from 4 in 2018 to 6 in 2019, 10 in 2020, 13 in 2021, and 15 in 2022. The discernible trend in the increasing number of reads, citations, and h-index values since 2018 is noteworthy.

In light of these data, it fills us with pride on behalf of the entire journal team to state that our dedicated efforts over the past three years have propelled our scientific publication to great heights, with its international reputation growing steadily.

Looking ahead to 2024, we are committed to continuing our efforts and striving to elevate our scientific publication, a national treasure, to the esteemed position it deserves.

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

Clinical Investigation / Araştırma

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Turk J Obstet Gynecol 2023;20:249-54

Predictive score for postpartum hemorrhage in vaginal deliveries following frozen embryo transfer

Dondurulmuş embriyo transferini takiben vajinal doğumlarda postpartum kanama için tahmin skoru

Akitoshi Yamamura, Akiko Okuda, Akiko Abe, Yuki Kashihara, Ayako Moribe, Yuki Kozono, Kentaro Sekiyama, Yumiko Yoshioka, Toshihiro Higuchi

Medical Research Institute Kitano Hospital, PIIF Tazuke-kofukai, Department of Obstetrics and Gynecology, Osaka, Japan

Abstract

Objective: To develop a predictive score for life-threatening severe postpartum hemorrhage in vaginal deliveries following frozen embryo transfer.

Materials and Methods: We conducted a retrospective cohort study of 315 singleton vaginal deliveries following frozen embryo transfer from 2017 to 2022. Severe postpartum hemorrhage was defined as hemorrhage exceeding 1500 mL. A predictive score was generated from maternal characteristics and obstetric complications before delivery. We performed multivariable logistic regression analysis using 2017-2020 data and assigned points to identified risk factors. The predictive score's accuracy was evaluated using 2021-2022 data.

Results: A large baby (birth weight ≥3500 g), pre-delivery maternal body mass index ≥25 kg/m^2, marginal or velamentous umbilical cord insertion, and history of postpartum hemorrhage were identified as risk factors. We assigned one point to a large baby, a pre-delivery maternal body mass index ≥25 kg/m^2, and marginal or velamentous umbilical cord insertion, and two points to a history of postpartum hemorrhage. The sum of the points was defined as the predictive score. The cut-off was set at two points, with a score ≥2 points being the high-risk group and a score ≤1 point being the low-risk group. The predictive score demonstrated a sensitivity of 47.8%, specificity of 85.4%, positive predictive value of 45.8%, and negative predictive value of 86.4% in the 2021-2022 validation cohort.

Conclusion: The predictive score identified severe postpartum hemorrhage in approximately half of the high-risk cases. Implementing measures such as autologous blood storage may facilitate rapid response during heavy bleeding and improve maternal prognosis.

Keywords: Postpartum hemorrhage, reproductive techniques, assisted, embryo transfer, risk factors

Öz

Amaç: Bu çalışmanın amacı dondurulmuş embriyo transferini takiben vajinal doğumlarda yaşamı tehdit eden ciddi pospartum kanama için öngörücü bir skor geliştirmektir.

Gereç ve Yöntemler: 2017'den 2022'ye kadar donmuş embriyo transferini takiben 315 tekil vajinal doğumun dahil edildiği retrospektif bir kohort çalışması gerçekleştirdik. Postpartum ciddi kanama, 1,500 mL'yi aşan kanama olarak tanımlandı. Doğumdan önce annenin özelliklerinden ve obstetrik komplikasyonlardan tahmin skoru oluşturuldu. 2017-2020 verilerini kullanarak çok değişkenli lojistik regresyon analizi yaptık ve belirlenen risk faktörlerine puan verdik. Tahmin puanının doğruluğu 2021-2022 verileri kullanılarak değerlendirildi.

Bulgular: Bebeğin iri olması (doğum ağırlığı ≥3,500 g), doğum öncesi annenin vücut kitle indeksinin ≥25 kg/m^2 olması, marjinal veya velamentöz göbek kordonu takılması ve postpartum kanama öyküsünün olması risk faktörleri olarak belirlendi. İri bebeğe, doğum öncesi annenin vücut kitle indeksinin ≥25 kg/m^2 olmasına ve marjinal veya velamentöz göbek kordonu takılmasına birer puan, postpartum kanama öyküsüne ise iki puan verdik. Puanların toplamı tahmin puanı olarak tanımlandı. Kesme noktası olarak iki puan olarak belirlendi ve alınan puana göre iki grup oluşturuldu: ≥2 puan yüksek riskli grup, ≤1 puan ise düşük riskli grup. Tahmin skoru, 2021-2022 doğrulama kohortunda %47,8 duyarlılık, %85,4 özgüllük, %45,8 pozitif prediktif değer ve %86,4 negatif prediktif değer gösterdi.

PRECIS: This study developed a score predicting over 1500 mL hemorrhage in vaginal deliveries after frozen embryo transfer with 47.8% sensitivity and 45.8% positive predictive value.

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Sonuç: Tahmin skoru, yüksek riskli olguların yaklaşık yarısında şiddetli postpartum kanamayı öngörebildi. Otolog kan depolama gibi önlemlerin uygulanması, ağır kanama sırasında hızlı müdahaleyi kolaylaştırabilir ve annenin prognozunu iyileştirebilir.

Anahtar Kelimeler: Postpartum kanama, üreme teknikleri, yardımlı, embriyo transferi, risk faktörleri

Introduction

Postpartum hemorrhage (PPH) is a significant global health concern because severe PPH remains one of the primary causes of maternal mortality[1-3]. Timely intervention is crucialbecause a delayed response can lead to increased mortality rates[4]. By predicting severe PPH in advance, appropriate preparations can be made, such as autologous blood storage.

Although developed countries have observed a decline in severe PPH-related maternal deaths, the overall incidence of PPH is reportedly on the rise[5,6]. This increase has been attributed to the growing number of pregnant women with PPH risk factors, including advanced maternal age, nulliparity, placenta previa, placenta accreta spectrum, and pregnancies achieved by assisted reproductive technology (ART)[7-14]. Several studies have examined the relationship between ART and PPH, with some focusing on the incidence of PPH in frozen embryo transfers compared with fresh embryo transfers[13,14].

Our previous research uncovered a notably high incidence of PPH in vaginal deliveries resulting from pregnancies achieved by frozen embryo transfer[15]. In that study, PPH in vaginal deliveries of singleton fetuses was defined as blood loss of 800 mL or more, based on the 90th percentile of blood loss[15]. Approximately half of the vaginal deliveries following frozen embryo transfers met this definition although blood loss of 800 mL rarely necessitates special intervention. Predicting transfusion-requiring blood loss could improve maternal outcomes.

To date, while research has been conducted to assess PPH risk using three-tiered systems, such as the California Maternal Quality Care Collaborative (CMQCC) admission hemorrhage risk score, there has been limited investigation into predictive scores specifically for severe PPH[16]. In this study, we aimed to develop a predictive score capable of identifying a high-risk population, namely those at risk of experiencing hemorrhage of 1500 mL or more in singleton vaginal deliveries following frozen embryo transfer.

Materials and Methods

Data Extraction

We conducted a retrospective cohort study using data from women who underwent vaginal delivery following pregnancies achieved by frozen embryo transfer. This included both embryo transfers in a natural cycle and those in a hormone replacement therapy cycle. Data were collected from delivery records at Kitano Hospital between January 1, 2017, and December 31, 2022. The hospital serves as a regional perinatal center, with approximately 700 deliveries annually and a cesarean section rate of approximately 25%. All singleton pregnancies achieved using the aforementioned methods and delivered vaginally after 22 weeks of gestation were included in the study, totaling 315 cases.

Outcome Definition

Severe PPH was defined as hemorrhage exceeding 1500 mL, which corresponds to stage 3 of the CMQCC staging system[16]. The actual amount of blood loss during vaginal delivery was determined by collecting blood with gauze immediately after delivery of the baby until 2 h post-placenta delivery and subsequently measuring the weight of the gauze.

Selection and Definition of Potential Risk Factors

A literature review and hypothesis generation guided the selection of potential risk factors for severe PPH, which were then subjected to statistical analysis. Factors known or predictable before delivery included large baby (birth weight over 3500 g), gestational diabetes mellitus or overt diabetes mellitus during pregnancy, hypertensive disorder of pregnancy, complication of leiomyoma, marginal or velamentous umbilical cord insertion, body mass index (BMI) of 25 kg/m^2 or higher just before delivery, advanced maternal age (over 35), history of uterine surgery, nulliparity, and history of PPH. Additional complications, such as clinical chorioamnionitis, premature rupture of the membrane, induction of labor, and placenta accreta spectrum (PAS), were also considered but excluded from predictive score calculations because they were only known immediately before or after delivery.

In relation to the term "large baby", macrosomia is traditionally defined as a birth weight over 4000 g. However, there has been a notable decline in birth weight in Japan in recent years[17]. Consistent with this trend, our study data revealed a limited number of cases that met the standard definition of macrosomia. To effectively identify cases with an elevated risk of severe PPH, we established a criterion for "large baby" as a birth weight over 3500 g.

Consistent with our prior study, a history of PPH was defined as a previous hemorrhage exceeding the 90th percentile of blood loss per mode of delivery and number of fetuses, according to the Perinatal Committee of the Japanese Society of Obstetrics and Gynecology report[18]. The 90th percentile of blood loss for vaginal delivery was 800 mL for singleton and 1500 mL for twin deliveries. No cases of vaginal birth after cesarean section were recorded. In instances where previous deliveries occurred at other hospital and blood loss data were unavailable, histories of PPH were assumed to exist when blood transfusions were performed. PAS cases included those clinically or pathologically diagnosed.

Ethical Considerations

The study received approval from the Ethics Committee of Kitano Hospital (date: 5/16/2023, number: 2205005) and was conducted in adherence to the Declaration of Helsinki and other pertinent Japanese laws and regulations. Written informed consent was obtained.

Statistical Analysis

Statistical analyses were performed using R version 4.1.1 and EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria)[19]. EZR is a modified version of the R commander, specifically designed to incorporate frequently used biostatistical functions.

Initially, maternal characteristics and obstetric complications were stratified by the presence or absence of severe PPH in the 2017-2020 and 2021-2022 datasets, respectively. Subsequently, using the 2017-2020 dataset from our previous study, a multivariate logistic regression analysis was conducted with potential risk factors to predict severe PPH before delivery. Variables were meticulously selected on the basis of analysis results and clinical relevance. The selected risk factors were assigned points according to their odd ratios, and a predictive score was calculated.

The accuracy of the score in predicting severe PPH was first assessed in the 2017-2020 dataset, and its predictive accuracy was then validated using the 2021-2022 dataset.

We used the strengthening the reporting of observational studies in epidemiology statement in reporting this study[20].

Results

First, maternal characteristics and obstetric complications were stratified by the presence or absence of severe PPH in the 2017-2020 and 2021-2022 datasets, respectively. The incidence of severe PPH was 15.3% in the 2017-2020 data and 20.5% in the 2021-2022 data. Although p-values were not calculated to avoid multiple testing, a trend emerged where cases with severe PPH in both the 2017-2020 and 2021-2022 datasets were more likely to have a large baby, marginal or velamentous insertion of the umbilical cord, a pre-delivery maternal BMI over 25, and a history of PPH (Table 1).

The patient analysis flow is depicted in Figure 1. A multivariate logistic regression analysis was conducted on the 2017-2020 data, focusing on factors known or predictable before delivery to identify risk factors for severe PPH (Table 2). Consistent with the univariate analysis, a large baby, marginal or velamentous insertion of the umbilical cord, pre-delivery maternal BMI over 25, and history of PPH appeared to correlate with severe PPH. Considering the odds ratio, we assigned two points to the history of PPH and one point to the other factors. The predictive score was defined as the sum of these points. To ensure robustness, we also performed a multivariate logistic regression analysis for all cases in the 2017-2022 period, which revealed the same trend as the 2017-2020 data (Supplementary Table 1).

The distribution of the predictive score in the 2017-2020 cohort is shown in Table 3. The area under the curve (AUC) of the receiver operating characteristic (ROC) curve was 0.713. The cut-off was set at two points, with a score ≥2 points being the high-risk group and a score ≤1 point being the low-risk group. Sensitivity was 46.4%, specificity was 89.0%, positive predictive value was 43.2%, and negative predictive value was 90.2%.

Table 1. Background characteristics and complications of eligible patients

	2017-2020		2021-2022	
	Severe PPH Yes (n=31)	No (n=172)	Severe PPH Yes (n=23)	No (n=89)
Large baby, n (%)	10 (32.3)	26 (15.1)	7 (30.4)	12 (13.5)
GDM, n (%)	2 (6.5)	12 (7.0)	2 (8.7)	5 (5.6)
HDP, n (%)	2 (6.5)	7 (4.1)	3 (13.0)	5 (5.6)
ICSI, n (%)	15 (48.4)	79 (45.9)	12 (52.2)	57 (64.0)
Complication of leiomyoma, n (%)	6 (19.4)	21 (12.2)	2 (8.7)	10 (11.2)
Marginal cord insertion, n (%)	7 (25.0)	19 (11.0)	6 (26.1)	10 (11.2)
Advanced maternal age, n (%)	23 (74.2)	130 (75.6)	17 (73.9)	68 (76.4)
History of PPH, n (%)	3 (9.7)	1 (0.6)	6 (26.1)	8 (9.0)
History of uterine surgery, n (%)	3 (9.7)	15 (8.7)	6 (26.1)	22 (24.7)
Nulliparity, n (%)	19 (61.3)	116 (67.4)	15 (65.2)	58 (65.2)
Induction of labor, n (%)	22 (71.0)	90 (52.3)	18 (78.3)	45 (50.6)
Placenta accreta spectrum, n (%)	11 (35.5)	3 (1.7)	8 (34.8)	3 (3.4)
Pre-delivery maternal BMI ≥25, n (%)	18 (58.1)	55 (32.0)	14 (60.9)	30 (33.7)
PROM, n (%)	10 (33.3)	67 (39.0)	9 (39.1)	25 (28.1)

GDM: Gestational diabetes mellitus, HDP: Hypertensive disorder of pregnancy, ICSI: Intracytoplasmic sperm injection, PPH: Postpartum hemorrhage, BMI: Body mass index, PROM: Premature rupture of membranes, Large baby: Birth weight ≥3500 g, GDM: Gestational diabetes mellitus or overt diabetes mellitus during pregnancy, Marginal cord insertion: Marginal or velamentous insertion of the umbilical cord, Advanced maternal age: ≥35 years old at the time of delivery, History of PPH: ≥800 mL for singleton vaginal delivery or ≥1500 mL for twins

The accuracy of the predictive scores was validated using the 2021-2022 data, yielding the following results (Table 4). The distribution of the predictive score was similar to that of the 2017-2020 data. The AUC of the ROC curve was 0.751, with a sensitivity of 47.8%, specificity of 85.4%, positive predictive value of 45.8%, and negative predictive value of 86.4%.

The distribution of hemorrhage according to the predictive score was expressed as a box and whisker plot for the 2017-2020 and 2021-2022 datasets, respectively (Figure 2). In each dataset, the high-risk group with a score of two or more tended to have a larger bleeding volume. However, even in the low-risk group with a score of 0 or 1, there were often outliers with a substantial amount of bleeding.

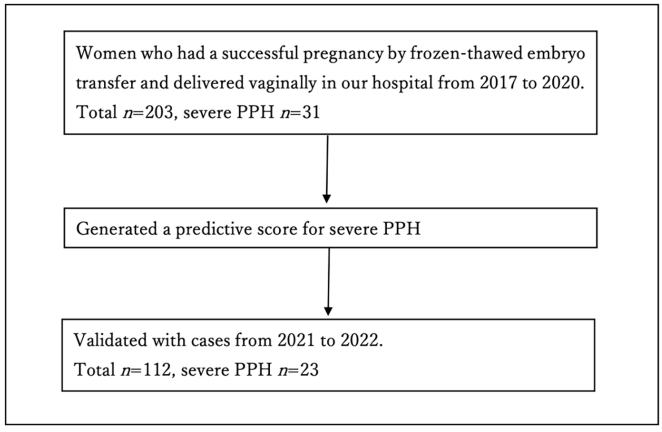

Figure 1. Patient analysis flow chart. Patients were analyzed as this flow

PPH: Postpartum hemorrhage

Discussion

We found that cases of severe PPH were more likely to have a large baby, marginal or velamentous insertion of the umbilical cord, pre-delivery maternal BMI over 25, and a history of PPH, with these associations remaining after adjusting for other factors. A predictive score was developed and validated using a validation cohort, achieving a sensitivity of 47.8% and a positive predictive value of 45.8%.

In our study population, 17.1% of singleton vaginal deliveries following frozen-thawed embryo transfer experienced hemorrhages of 1500 mL or more. The incidence of obstetrical hemorrhage is 13.6% in populations classified as high-risk according to the CMQCC score[21]. In that study, obstetrical hemorrhage was defined as bleeding of 1000 mL or more, regardless of the mode of delivery, according to the American College of Obstetricians and Gynecologists definition. This demonstrates how high-risk the population in our study is, specifically those who achieved pregnancy through frozen-thawed embryo transfer and underwent vaginal delivery. By developing a predictive score within this high-risk population, we achieved a higher positive predictive value for severe PPH occurrence than previously reported.

In addition to the report that frozen-thawed embryo transfers increase PAS, our previous study indicated that minor placental adhesions not diagnosed as PAS may also increase[15,22]. These factors may have contributed to the unusually high incidence of severe PPH in our study population. Patients with a predictive score of two or higher have approximately a 50% risk of experiencing severe PPH, suggesting that precautions such as advanced autologous blood storage should be considered.

Table 2. Risk factors for severe PPH result of multivariable logistic regression analysis with 2017-2020 data and development of the predictive score

Risk factor	Odds ratio	95% CI	p-value	Points
Complication of leiomyoma	1.30	0.38-4.48	0.67	0
Advanced maternal age	0.63	0.21-1.89	0.41	0
GDM	0.90	0.15-5.61	0.91	0
HDP	1.34	0.19-9.39	0.77	0
History of PPH	16.10	1.16-223.00	0.04	2
History of uterine surgery	0.68	0.11-4.05	0.67	0
ICSI	0.94	0.38-2.31	0.89	0
Large baby	2.84	1.02-7.93	0.05	1
Marginal cord insertion	3.33	1.11-10.00	0.03	1
Nulliparity	0.85	0.31-2.33	0.75	0
Pre-delivery maternal BMI ≥25	2.53	0.97-6.61	0.06	1

Predictive score: The sum of the points, GDM: Gestational diabetes mellitus, HDP: Hypertensive disorder of pregnancy, ICSI: Intracytoplasmic sperm injection, PPH: Postpartum hemorrhage, BMI: Body mass index, Large baby: Birth weight ≥3500 g, GDM: Gestational diabetes mellitus or overt diabetes mellitus during pregnancy, Marginal cord insertion: Marginal or velamentous insertion of the umbilical cord, Advanced maternal age: ≥35 years old at the time of delivery, History of PPH: ≥800 mL for singleton vaginal delivery or ≥1500 mL for twins

Table 3. Relationship between predictive score and severe PPH with 2017-2020 data

Predictive score	Severe PPH Yes (n=31)	No (n=172)
0	7	90
1	8	63
2	9	18
3	3	1
4	1	0
5	0	0

AUC of ROC curve: 0.713 (0.602-0.823)
When two points are cut-off;
Sensitivity: 0.464
Specificity: 0.890
Positive predictive value: 0.432
Negative predictive value: 0.902
PPH: Postpartum hemorrhage, AUC: Area under the curve, ROC: Receiver operating characteristic

Table 4. Validation of the predictive score with 2021-2022 data

Predictive score	Severe PPH Yes (n=23)	No (n=89)
0	2	41
1	10	35
2	6	7
3	3	5
4	2	1
5	0	0

AUC of ROC curve: 0.751 (0.65-0.852)
When two points are cut-off;
Sensitivity: 0.478
Specificity: 0.854
Positive predictive value: 0.458
Negative predictive value: 0.864
PPH: Postpartum hemorrhage, AUC: Area under the curve, ROC: Receiver operating characteristic

Study Limitations

Although this predictive score does provide some indication of severe PPH occurrence, it failed to predict over half of the cases that developed into severe PPH. In addition to the risk factors incorporated in this predictive score, other risk factors for PPH known before delivery, such as nulliparity, hypertensive disorder of pregnancy, and advanced maternal age, have been reported[7-9]. Although this was a single-center study, conducting a larger, multicenter study to incorporate these factors into a more comprehensive predictive score may enable more accurate prediction.

This study focused on cases of pregnancies achieved by frozen-thawed embryo transfer with a high probability of severe PPH. However, severe PPH can also occur in natural pregnancies. Future research should develop a prediction score for severe PPH without limiting the study population.

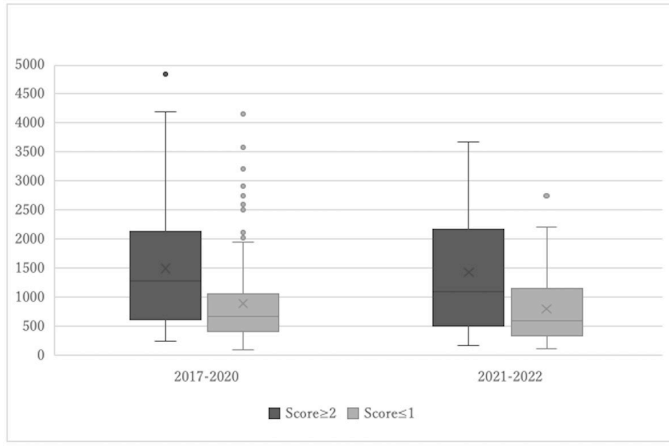

Figure 2. Distribution of hemorrhage by predictive score. Both the 2017-2020 data and the 2021-2022 validation cohort showed a trend toward more bleeding in the high-risk group with a predictive score ≥2 points

Conclusion

In cases of vaginal deliveries following pregnancies achieved by frozen-thawed embryo transfer, nearly half of the patients with a risk score of two or higher experienced severe PPH. Consequently, we recommend that patients with a risk score of two or higher undergo advanced autologous blood storage. Future large-scale, multicenter studies could develop a more generalized risk score for severe PPH, contributing to safer delivery practices.

Ethics

Ethics Committee Approval: The study received approval from the Ethics Committee of Kitano Hospital (date: 5/16/2023, number: 2205005) and was conducted in adherence to the Declaration of Helsinki and other pertinent Japanese laws and regulations.

Informed Consent: Written informed consent was obtained.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: A.Y., A.O., A.A., Y.K., A.M., Y.Ko., K.S., Y.Y., T.H., Concept: A.Y., A.O., T.H., Design: A.Y., A.O., T.H., Data Collection or Processing: A.Y., A.A., Y.K., Analysis or Interpretation: A.Y., A.M., Y.Ko., K.S., Y.Y., T.H., Literature Search: A.Y., A.O., T.H., Writing: A.Y.

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Supplementary Table 1. Result of multivariable logistic regression analysis with 2017-2022 data

Risk factor	Odds ratio	95% CI	p-value
Complication of leiomyoma	1.08	0.42-2.75	0.87
Advanced maternal age	0.72	0.34-1.54	0.39
GDM	0.86	0.25-2.96	0.81
HDP	1.81	0.54-6.07	0.33
History of PPH	6.35	1.95-20.60	<0.01
History of uterine surgery	1.11	0.44-2.76	0.83
ICSI	0.87	0.46-1.67	0.68
Large baby	3.06	1.46-6.42	<0.01
Marginal cord insertion	3.23	1.43-7.29	<0.01
Nulliparity	1.42	0.64-3.14	0.38
Pre-delivery maternal BMI ≥25	2.61	1.34-5.10	<0.01

GDM: Gestational diabetes mellitus, HDP: Hypertensive disorder of pregnancy, ICSI: Intracytoplasmic sperm injection, PPH: Postpartum hemorrhage, BMI: Body mass index, Large baby: Birth weight ≥3500 g, GDM: Gestational diabetes mellitus or overt diabetes mellitus during pregnancy, Marginal cord insertion: Marginal or velamentous insertion of the umbilical cord, Advanced maternal age: ≥35 years old at the time of delivery, History of PPH: ≥800 mL for singleton vaginal delivery or ≥1500 mL for twins

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Assessing lipoxin-mediated inflammatory responses in the second trimester of pregnancy among women with obesity: A comprehensive analysis

Obezite ile komplike gebeliklerde ikinci trimester lipoksin aracılı enflamatuvar yanıtların değerlendirilmesi

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Abstract

Objective: This study aimed to explore the relationship between maternal plasma lipoxin A4 (LXA4) levels during the second trimester of pregnancy and certain proinflammatory molecules, such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α), as well as the antiangiogenic factor vascular endothelial growth factor receptor 1 (VEGFR-1), in conjunction with obesity among pregnant women.

Materials and Methods: A total of 30 pregnant women with obesity were compared with 30 pregnant women of normal weight, matched for both age and gestational week. Plasma samples were collected from all participants between the 18th and 28th weeks of pregnancy. The levels of LXA4, VEGFR-1, IL-6, and TNF-α were quantified using enzyme-linked immunosorbent assay.

Results: Plasma levels of LXA4 were notably lower in pregnant women with obesity, whereas levels of TNF-α and VEGFR1 were significantly higher ($p=0.041$, $p<0.001$, and $p<0.001$, respectively). There was no significant difference in IL-6 levels between groups ($p=0.072$). The binary logistic regression model revealed significant associations between obesity and the examined inflammatory mediators. Specifically, the results demonstrated that higher levels of LXA4 were linked to a reduced obesity risk, with each unit increase corresponding to a 0.926-fold decrease in the likelihood of obesity. Conversely, elevated levels of TNF-α and VEGFR1 were associated with an increased risk of obesity.

Conclusion: The study concluded that increased body mass index during pregnancy affects the levels of plasma lipoxin, cytokines, and angiogenesis-related factors. Although the exact mechanisms remain unclear, the observed changes suggest a disruption in the metabolic systems of women with obesity, which may influence physiological changes during pregnancy and lead to obesity-related pathological conditions.

Keywords: Angiogenic and antiangiogenic factors, inflammatory mediators, lipoxins, maternal obesity, pregnancy complications, vascular endothelial growth factor receptor 1

Öz

Amaç: Bu araştırmanın amacı, maternal obezite ile komplike gebelerde ikinci trimester maternal plazma lipoksin A4 (LXA4) düzeyleri ile interlökin-6 (IL-6) ve tümör nekroz faktör alfa (TNF-α) gibi proenflamatuvar moleküller ile birlikte antianjiyogenik faktör vasküler endotelyal büyüme faktörü reseptörü 1 (VEGFR-1) arasındaki ilişkiyi aydınlatmaktır.

Gereç ve Yöntemler: Çalışmaya obezite ile komplike 30 gebe ile yaş ve gebelik haftası açısından eşleştirilmiş 30 normal kilolu gebe dahil edildi. Katılımcılardan gebeliğin 18 ila 28. haftaları arasında plazma örnekleri toplandı. LXA4, VEGFR-1, IL-6 ve TNF-α seviyeleri, enzime bağlı immünosorbent testi kullanlarak ölçüldü.

PRECIS: Pregnant women with obesity show decreased plasma LXA4 levels and increased TNF-α and VEGFR-1 levels, indicating associations with maternal obesity risks and suggesting potential disruptions in metabolic systems during pregnancy.

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Bulgular: Obezite ile komplike gebe kadınların plasma LXA4 seviyeleri anlamlı derecede düşük, TNF-α ve VEGFR-1 plazma düzeyleri ise anlamlı derecede yüksek saptandı (sırasıyla p=0,041, p<0,001 ve p<0,001). Gruplar arasında IL-6 düzeyleri açısından anlamlı fark izlenmedi (p=0,072). Binary lojistik regresyon modeli, obezite ve incelenen enflamatuvar mediatörler arasında anlamlı ilişki olduğunu ortaya çıkardı. Spesifik olarak, sonuçlar, daha yüksek LXA4 seviyelerinin obezite riskinin azalmasıyla bağlantılı olduğunu ve her birim artışın obezite olasılığında 0,926 katlık bir azalmaya karşılık geldiğini gösterdi. Tersine, yüksek TNF-α ve VEGFR-1 düzeylerinin ikisi de obezite riskinin artmasıyla ilişkilendirildi.

Sonuç: Çalışma, gebelikte artan vücut kitle indeksinin plazma lipoksin, sitokin ve anjiyogenez ile ilişkili faktörleri etkilediği sonucuna varmıştır. Kesin mekanizmalar belirsizliğini korusa da, gözlemlenen değişikliklerin obezite ile komplike kadınların metabolik sistemlerinde, gebelik sırasındaki fizyolojik değişiklikleri etkileyebilecek ve obezite ile ilişkili patolojik durumlara yol açabilecek bozulma ile ilişkili olduğunu göstermektedir.

Anahtar Kelimeler: Anjiyojenik ve antianjiyogenik faktörler, enflamatuvar mediatörler, lipoksinler, maternal obezite, gebelik komplikasyonları, vasküler endotelyal büyüme faktörü reseptörü 1

Introduction

Pregnancy-associated obesity is a major public health concern that poses acute and chronic risks to both maternal and neonatal well-being[1]. Such obesity is characterized by a body mass index (BMI) greater than 30 kg/m^2 recorded at the initial antenatal assessment. Based on current data, approximately 30-70% of adults in Europe have excess weight, with 10-30% classified as having obesity[2]. Alarmingly, the global incidence of obesity is increasing rapidly and is approaching pandemic proportions. Evidence shows that maternal obesity increases both immediate health complications and mortality, and this risk profile extends to the long-term health prospects of both mothers and children. An estimated 24% of all pregnancy complications are due to maternal overweight or obesity[3]. In addition, excessive gestational weight gain is associated with one-third of all large-for-gestational-age (LGA) neonates. Pregestational obesity is associated with reduced fertility and a variety of pregnancy complications, including miscarriage, thromboembolism, gestational diabetes mellitus (GDM), hypertension, preeclampsia, congenital fetal anomalies, preterm birth, macrosomia, and postterm delivery. In addition, maternal obesity associated with pregnancy complications increases the long-term risk of obesity, diabetes, and cardiovascular disease in offspring[4]. These compelling statistics underscore the urgent need for a thorough understanding of the biological underpinnings that contribute to adverse perinatal outcomes in pregnancies complicated by obesity.

Although a limited number of studies have investigated maternal systemic inflammation in pregnant women with obesity, their findings are often conflicting. It has been postulated that physiological adaptations associated with pregnancy may mask the underlying inflammatory responses attributable to obesity[5]. Because of the significant involvement of inflammation in the development of both obesity and pregnancy, it is conceivable that collaborative interactions between maternal physiological adaptations and the inflammatory responses triggered by obesity could lead to an excessive amplification of inflammatory mediators. This, in turn, could contribute to an increase in both immediate and long-term morbidity in pregnant women with obesity. The expansion of adipose tissue contributes to the infiltration of macrophages and the release of inflammatory adipokines, including leptin, tumor necrosis factor alpha (TNF-α), and interleukin-6 (IL-6)[6]. In obesity, there is an overexpression of TNF-α and IL-1β, which hampers insulin signaling in both animal and human adipose tissue. TNF-α, primarily a proinflammatory cytokine produced by myeloid cells, triggers the release of other inflammatory cytokines such as IL-6 and IL-1β. IL-6, primarily secreted by adipocytes, is linked to conditions such as hyperglycemia, insulin resistance, and obesity[7]. As with TNF-α, increases in body mass and waist circumference lead to increased production of IL-6 relative to free fatty acids. Within the context of maternal obesity, this condition manifests as an inflammatory metabolic disorder characterized by increased circulating proinflammatory cytokines and greater macrophage accumulation in adipose tissue. Inflammation also affects the placenta, creating an intrauterine environment that is prone to inflammation. In addition, research has found a positive correlation between maternal serum levels of IL-6 and fetal growth, linking the proinflammatory state of mothers with obesity to excessive intrauterine growth[8]. Although higher levels of cytokines such as leptin, C-reactive protein (CRP), IL-6, and intercellular adhesion molecule-1 have been found in pregnancies affected by obesity compared with similar pregnancies in individuals without obesity, these observations are not consistently replicated for all inflammatory markers[9]. The full picture of inflammatory mediators in pregnancies with obesity remains unclear and a subject of debate in the scientific community. Therefore, further research is needed to determine the medical relevance of these inflammatory molecules in the management of obesity in pregnancy.

Lipoxins (LXs) are bioactive lipid mediators synthesized from arachidonic acid with potent anti-inflammatory and immunoregulatory properties. LX, an endogenously produced eicosanoid, has anti-inflammatory, anabolic, and antifibrotic properties[10]. Lipoxins induce the inactivation of the major proinflammatory pathway and release of resoluble cytokines by downregulation proinflammatory cytokines and chemokines. Lipoxin A4 (LXA4) is a molecule that reduces adipose tissue inflammation and insulin resistance[11]. The natural lipid mediator LXA4 plays a crucial role in maintaining a healthy pregnancy by modulating factors related to inflammation, mast cells, and various other cellular components. It acts as a vital regulator in the complex biological processes of pregnancy,

contributing significantly to maintaining the delicate balance between inflammation and resolution necessary for a successful pregnancy[12]. Despite its pivotal role, there is a noticeable gap in the scientific literature regarding comprehensive investigations into the relationship between serum LXA4 levels and maternal obesity. Although it is well established that maternal obesity is linked to various adverse pregnancy outcomes and heightened inflammatory responses, the potential connections between these altered physiological and immunological states and variations in LXA4 levels remain poorly understood. This gap in understanding highlights the need for further investigation into this complex interplay of factors.

The successful achievement of an optimal pregnancy outcome relies on the establishment of the maternal– fetal vascular interface during early gestation and the continuous process of placentation throughout pregnancy. Disruptions in the placental production of angiogenic factors due to an imbalance in angiogenesis can lead to a range of adverse perinatal outcomes[13]. At the core of this vascular interface are the vascular endothelial growth factor (VEGF) and its receptor (VEGFR) system, which predominantly drives angiogenic activity within adipose tissue. VEGF family members bind to transmembrane tyrosine kinase receptors, specifically VEGFR1 (Flt-1), VEGFR2 (KDR/Flk-1), and VEGFR3 (Flt-4). Notably, VEGFR-1 and VEGFR-2 play significant roles as mediators of angiogenesis, whereas VEGFR-3 is involved in lymphangiogenesis regulation[14]. Imbalanced levels of certain antiangiogenic factors, including soluble fms-like tyrosine kinase 1 (sFlt1) and endoglin, have been strongly linked to various placental disorders such as preeclampsia, placental abruption, stillbirth, and intrauterine growth restriction. Recent studies have suggested that elevated maternal serum Flt1 levels may increase the risk of preterm birth, a risk that appears to be unrelated to pre-eclampsia[15]. It has been postulated that maternal obesity may induce an angiogenic imbalance via multiple adipokine-mediated pathways. However, our current understanding of the relationship between maternal obesity and the balance between angiogenic and antiangiogenic factors remains limited, particularly in the context of human pregnancy. Therefore, further investigation of this association is warranted to better understand its mechanistic basis and potential clinical implications.

Despite the literature documenting increased serum cytokine levels in pregnant women with obesity, there is a notable gap in our understanding of the relationship between serum LXA4 levels and maternal obesity. Therefore, this study aims to fill this knowledge gap. The primary objective of this research endeavor is to delineate the association between second trimester maternal plasma LXA4 levels, proinflammatory molecules IL-6 and TNF-α, and anti-angiogenic factor VEGFR-1 and obesity in pregnant women. The results of this investigation are expected to provide important insights into the dynamic interplay of these molecules in the context of maternal obesity.

Materials and Methods

The study encompassed all expectant mothers treated at the Inonu University Faculty of Medicine, Department of Obstetrics and Gynecology, during the period from May 01, 2021, to May 01, 2022, between the 18th and 28th weeks of gestation. Ethical approval was obtained from the Inonu University Clinical Research Ethics Committee (approval number: 2021/114, date: 31.03.2021). Adhering to the principles of the Declaration of Helsinki (2013 revision), the participants received comprehensive written and verbal details about the study, and their informed consent was duly obtained. Thirty pregnant women with a body mass index of 30 kg/m^2 and above formed the study group, whereas 30 normal-weight pregnant women with a body mass index between 18.5 and 24.9, matched for age and gestational weeks, constituted the control group. The gestational weeks of the pregnant women who participated in the study were confirmed based on the first trimester ultrasound measurements.

Participants were eligible for inclusion in the study if they met the following criteria:

- Women aged between 18 and 45 years.
- A singleton viable pregnancy.
- Normal obstetric and medical history with no history of any significant health issues.

Conversely, individuals were excluded from the study based on the following criteria:

- The presence of multiple pregnancies (twins, triplets, etc.).
- Evidence of coexisting systemic diseases in pregnant women, including chronic hypertension, dyslipidemia, asthma, chronic renal failure, malignancies, and any cardiac or pulmonary diseases.
- Detection of chromosomal abnormalities and fetal malformations.
- History of cigaret smoking and alcohol consumption during pregnancy.

Standard serum analyses were performed using the Abbott Architect C8000 system at the Biochemistry Laboratory of Inonu University School of Medicine. At the beginning of the study, 2 mL peripheral blood samples were collected from all participants as part of the routine laboratory procedure. These samples were collected in EDTA-anticoagulated tubes to prevent blood clotting. After collection, plasma was isolated by centrifugation at 3000 g for 15 min at room temperature and then stored at -80 °C to maintain sample integrity until analysis. After achieving the intended sample size, the frozen plasma samples were thawed. Quantitative levels of LXA4, VEGFR-1, IL-6, and TNF-α were determined using enzyme-linked immunosorbent assay (ELISA). Specifically, ELISA kits for LXA4, IL-6, TNF-α (catalog numbers E3155Hu, E2063Hu, and E0796Hu, respectively; manufactured by Sunredbio Corp, China), and VEGFR-1 (catalog number E3155Hu; manufactured by Cloude Clone Corp, China) were utilized, following the manufacturers' protocols. The measurement ranges for LXA4,

IL-6, TNF-α, and VEGFR-1 assays were 0.1-38.0, 1-400, 0.5-150, and 0.3-90.0 ng/mL, respectively. The inter- and intra-assay precision coefficients of variation consistently remained below 10% and 8%, respectively, ensuring the reliability and reproducibility of the results obtained from the ELISA kits used in this study.

In addition to the meticulous serum analyses, a comprehensive set of demographic, clinical, and biochemical parameters was systematically recorded for each participant.

Sample size calculation: The sample size calculation was based on a power analysis assuming that a 1.0 pg/mL decrease in the LXA4 ratio (equivalent to 1.7 standard deviations) in pregnant women with obesity would have a statistically significant effect. To detect such an effect with 80% power and a 5% significance level (two-tailed), a minimum of 30 participants would be needed in each study group.

Statistical Analysis

The statistical analysis was performed using SPSS software, version 22.0 (SPSS Inc, New York, USA). Baseline data for both the study and control groups are presented as medians with corresponding ranges and/or interquartile ranges for categorical variables. Continuous data are expressed as means, standard deviations, and minimum and maximum values. Data distribution was assessed using the Shapiro-Wilk test. Initial group comparisons involved two-sample t-tests for normally distributed data and Mann-Whitney U tests for non-normally distributed data. Categorical variables are indicated as counts and percentages, and comparisons were conducted using Pearson's exact chi-square and continuity-corrected chi-square tests. A binary logistic regression model was constructed, with group classification as the dependent variable and serum concentrations of LXA4 (ng/mL), IL-6 (ng/mL), TNF-α (ng/mL), and VEGFR1 (pg/mL) as independent variables. The "Enter" method was employed to simultaneously evaluate the significance of all variable coefficients in a single step. The goodness of fit of the model was assessed using the Hosmer-Lemeshow test, and a significance level of α=0.05 was considered for a two-tailed p-value.

Results

Clinical Characteristics of the Study Population

The statistical analysis revealed that there were no notable variations between the study and control groups in terms of age, gravidity, parity, gestational age at screening, gestational age at birth, mode of delivery, and birth weight (with p-values of 0.801, 0.079, 0.101, 0.250, 0.881, 0.639, and 0.115, respectively, as indicated in Table 1). Conversely, the obesity group exhibited significantly higher current and prepregnancy BMI levels, along with a higher occurrence of adverse perinatal outcomes compared with the control group (p-values of <0.001, <0.001, and 0.025, respectively, as presented in Table 1).

Evaluation of Plasma Levels of LXA4 (ng/mL), IL-6 (ng/mL), TNF-α (ng/mL) and VEGFR1 (pg/mL) in Pregnant Women with Obesity

Both pro- and anti-inflammatory mediators were assessed in the peripheral blood plasma of both the study and control groups. When compared with the control group, pregnant women with obesity showed a significant reduction in their plasma LXA4 concentration (p=0.041). No significant differences in IL-6 levels were observed between the two groups (p=0.072). In contrast, plasma levels of TNF-α and VEGFR1 were notably higher in the obesity group than in the control group (p<0.001 for both). Comprehensive information regarding the plasma concentrations of LXA4, IL-6, TNF-α, and VEGFR1 in both the study and control groups can be found in Table 2. Figure 1 illustrates the distribution of proinflammatory molecules in plasma samples from the study and control groups.

Predictive Value of Inflammatory Mediators in Obesity

To evaluate the capability of plasma proinflammatory molecules to predict obesity, we employed a binary logistic regression model. In this model, serum levels of LXA4, IL-6, TNF-α, and VEGFR-1 served as independent variables, whereas obesity (as opposed to control) was the dependent variable. The model's accuracy was confirmed through the Hosmer-Lemeshow test, indicating its capability to distinguish between the obesity and control groups (Hosmer-Lemeshow χ^2 =9.854, p=0.275>0.05). Our logistic regression model was statistically significant and reliable. In particular, an elevation of one unit (ng/mL) in LXA4 was associated with a 0.926-fold decrease in the likelihood of obesity. Likewise, a single unit increase in TNF-α corresponded to a 1.026-fold increase in the risk of obesity (ng/L), whereas an increment in VEGFR1 was linked to a 1.003-fold increase in the odds of obesity (ng/mL). Further details, such as parameter estimates (β), standard errors (se), Wald statistics (W), degrees of freedom (df), odds ratios [Exp (β)], and 95% confidence intervals, are available in Table 3.

Discussion

This study provides a substantial contribution to the scientific discourse surrounding the assessment of obesity during pregnancy, particularly in the context of its association with serum LXA4, TNF-α and VEGFR1 levels. Although obesity and inflammation have been frequently discussed in recent scientific discourse, there remains a notable gap in our knowledge of the behavior of inflammatory and anti-inflammatory molecules in normal-weight women compared with those characterized as overweight during pregnancy. Despite the increased predisposition of obese women to placental vascular dysfunction, investigations into the pathophysiological factors that may contribute to this vulnerability remain limited. Accumulating evidence suggests that the regulation and effects of metabolic systems in individuals with obesity differ markedly from those of their normal weight counterparts[16]. LXA4, a prominent lipoxin

Table 1. Clinical characteristics and birth outcomes of the pregnant women with obesity and the control group

Variable			Obesity (n=30)	Control (n=30)	p-value
Age (years)*			33.5 (21-43)	33.1 (20-43)	0.801
Gravidity*			3 (1-7)	2 (1-6)	0.079
Parity*			2 (0-4)	1 (0-4)	0.101
Weight (kg) *			92 (72.5-107)	63 (46-80)	**<0.001**
Height (cm)*			162 (151-170)	160 (145-176)	0.710
BMI (kg/m²)*			35.2 (29.04-41.62)	23.86 (20.31-28.3)	**<0.001**
Gestational age at screening (weeks)*			25 (18-28)	27 (18-28)	0.250
Pregravid weight (kg)*			85.5 (65-105)	54.5 (47-65)	**<0.001**
Pregravid BMI (kg/m²)*			32.42 (27.06-39.52)	21.92 (17.8-28.55)	**<0.001**
Adverse perinatal outcomes**	**FGR**		1 (3.3)	3 (10)	**0.025**
	GHT/Preeclampsia		5 (16.7)	2 (6.7)	
	Preterm birth		2 (6.7)	0 (0)	
	PPROM		1 (3.3)	0 (0)	
	GDM		5 (16.7)	0 (0)	
	LGA		2 (6.7)	0 (0)	
Gestational age at birth (weeks)*			37 (26-41)	38 (28-40)	0.881
Mode of delivery**	**Vaginal delivery**		8 (26.7)	11 (36.7)	0.639
	Cesarean section		22 (73.3)	19 (63.3)	
Birthweight (g)*			3020 (875-4800)	2770 (755-3725)	0.115
Gender**		**Female**	17 (56.7)	17 (56.7)	1.000
		Male	13 (43.3)	13 (43.3)	
Cord blood pH***			7.37±0.17	7.30±0.15	0.118
Cord blood base excess***			-4.47±3.35	-5.28±4.54	0.600

* median (minimum-maximum); ** n (%); *** mean ± standard deviation
BMI: Body mass index, FGR: Fetal growth restriction, GHT: Gestational hypertension, PPROM: Preterm premature rupture of membranes, GDM: Gestational diabetes mellitus, LGA: Large for gestational age
p-values marked with bold indicate statistically significant differences between the groups

Table 2. Comparison of plasma levels of LXA4, IL -6, TNF-α, and VEGFR1 between the study and control groups

Variable	Obesity (n=30)	Control (n=30)	p-value
LXA4 (ng/mL)*	30 (14.42-79.42)	39 (15.01-71.34)	**0.041**
IL-6 (ng/L)*	16.26 (11.12-26.75)	13.73 (10.06-44.34)	0.072
TNF- α (ng/L)*	148.23 (103.83-196.26)	83.12 (49.26-153.35)	**<0.001**
VEGFR1 (pg/mL)*	632.19 (490.46-847.41)	487.31 (406.17-569.52)	**<0.001**

* median (minimum–maximum), LXA4: Lipoxin A4, IL: Interleukin, TNF: Tumor necrosis factor, VEGFR-1: Vascular endothelial growth factor receptor 1.
p-values marked with bold indicate statistically significant differences between the groups

in mammals, plays a critical role in regulating inflammation during the menstrual cycle, endometrial neuroregulation, embryo implantation, pregnancy, and parturition. An increase in LXA4 in the first trimester is associated with normal pregnancy and placental development, a decrease in the second trimester indicates a healthy pregnancy, and an increase in the third trimester is associated with normal pregnancy and preparation for delivery[17]. Endogenous LXA4 is a key determinant of normal

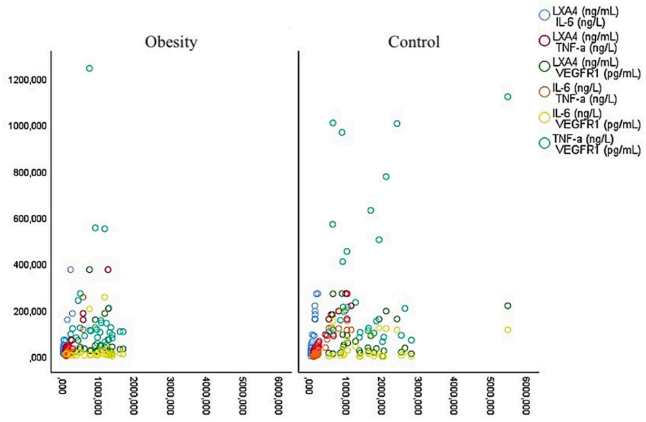

Figure 1. Binary distributions of parameters in the study and control groups

pregnancy outcomes by modulating mast cell migration and proinflammatory factors. Although a number of inflammatory markers have been analyzed previously, this study is the first to demonstrate significantly reduced plasma levels of LXA4 in the second trimester in pregnant women with obesity and is one of the first to demonstrate that factors associated with obesity may differentially influence aspects of inflammation and angiogenesis in overweight women compared with normal-weight women during pregnancy.

The receptor for LXA4, FPR2/ALX (N-formyl peptide receptor 2 and A lipoxin A), has been found to increase LXA4 levels in the third trimester of pregnancy relative to proinflammatory cytokines such as IL-1β and TNF-α[18]. Existing data on LXA4 levels during pregnancy are scarce. Previous studies have reported that LXA4 levels in women at 24 weeks of gestation exceed those in nonpregnant women[19]. Research by Perucci et al.[20] showed significantly elevated LXA4 levels in pregnant women with pre-eclampsia at or beyond 28 weeks gestation compared with normotensive pregnant women of the same gestational age. In this study, we found a significant difference in LXA4 levels in pregnant women between the obesity and control groups. The LXA4 levels observed in the pregnant women with obesity and the control group in our study are in line with the findings of Szczuko et al.[12], who documented variations in LXA4 levels throughout the weeks of pregnancy. Maternal obesity is an inflammatory metabolic disorder characterized by elevated circulating proinflammatory cytokines and increased macrophage infiltration within adipose tissue. This inflammation extends to the placenta, creating a proinflammatory intrauterine environment. Although there are documented cases of higher cytokine levels in pregnancies affected by obesity compared with pregnancies without obesity, these findings are not consistent across all inflammatory markers. The full picture of inflammatory mediators in pregnancies with obesity remains unclear and is the subject of ongoing debate within the scientific community. Therefore, further research is essential to determine the clinical significance of these inflammatory molecules in the management of obesity during pregnancy.

IL-6, a systemic adipokine, is secreted by adipose tissue and skeletal muscle in humans. Adipose tissue expansion is associated with an increase in the levels of proinflammatory adipokines, including TNF-α and IL-6[21]. In obesity, IL-6 has been implicated in the recruitment of macrophages into expanding adipose tissue, leading to chronic inflammation, impaired insulin sensitivity, and the potential development of type 2 diabetes[22]. TNF-α, secreted by macrophages in adipose tissue, is an inflammatory cytokine involved in the development and maintenance of insulin resistance[23]. In contrast to earlier stages of pregnancy, Friis et al.[24] found no significant variation in maternal IL-6 levels between BMI categories in pregnant women with obesity at 36-38 weeks of gestation and no significant difference in soluble tumor necrosis factor receptor II between BMI categories. Lodefalk et al.[25] reported a decrease in the expression of TNF, IL6, insulin-like growth factor (IGF)-1, and IGF2 in the placenta of pregnant women with obesity, which was inversely correlated with the duration of the pushing phase of labor. In pregnant patients with GDM and a BMI ≥33 kg/m^2, gene expression analysis of adipose tissue revealed significantly increased levels of TNF-α expression compared with controls[26]. Challier et al.[6] reported that plasma TNF-α levels in mothers with obesity were not significantly different from those in controls, whereas IL-6 and CRP levels were significantly elevated. In our study, no significant difference in

Table 3. Estimated values of the parameters in the model

Variables	β	SE	W	sd	p-value (sig)	Exp (β)	95% CI for Exp (β)	
							Lower limit	Upper limit
LXA4 (ng/mL)	-0.077	0.031	6.086	1	0.014	0.926	0.871	0.984
IL-6 (ng/L)	-0.002	0.018	0.014	1	0.907	0.998	0.964	1.034
TNF-α (ng/L)	0.026	0.009	9.032	1	0.003	1.026	1.009	1.044
VEGFR1 (pg/mL)	0.003	0.001	8.772	1	0.003	1.003	1.021	1.036
Constant	2.408	0.953	6.387	1	0.011	11.114		

β: parameter estimation, SE: standard error, W: Wald statistic, sd: degrees of freedom, Exp (β): odds ratio, 95% CI: confidence interval, LXA4: Lipoxin A4, IL: Interleukin, TNF: Tumor necrosis factor, VEGFR-1: Vascular endothelial growth factor receptor 1

IL-6 levels was observed between the obese and control groups, whereas a statistically significant increase in TNF-α levels was observed. Higher IL-6 levels in the early weeks of pregnancy in women with obesity probably reflect prepregnancy status rather than gestational weight gain[27]. Another study reported that IL-6 levels increased in the early weeks of pregnancy, decreased slightly in the middle of pregnancy, and increased again in the late weeks of pregnancy[28]. The lack of a significant difference in IL-6 levels in our study supports the conclusion that obesity in early pregnancy may have a greater influence on inflammation. The significantly elevated TNF-α levels in the group with obesity compared to normal TNF-α levels in pregnancy highlight the potentially pivotal role of TNF-α and LXA4 in modulating inflammatory processes within visceral adipose tissue.

Angiogenesis, which is associated with VEGF and placental growth factor levels, plays a critical role in the physiological and pathological conditions of embryonic development. VEGF mediates its effects through interaction with VEGFR-1/flt-1 and VEGFR-2/KDR receptors. In studies investigating conditions such as hypoxia, maternal sleep apnea, hyperglycemia, obesity, and abnormal fetal growth, changes in the expression levels of angiogenesis factors such as VEGF, VEGFR-1, and VEGFR-2 in plasma and placenta have been observed[29]. Dubova et al.[30] investigated placental VEGFR expression in both pregnancies with obesity and normal weight and found decreased VEGFR1 in the vascular endothelium, whereas VEGFR-2 and VEGFR-3 were increased in nonvillous cytotrophoblasts and endothelial cells of mature intermediate and terminal villous capillaries. In contrast, another study showed that preexisting obesity and diabetes had no significant effect on the expression or secretion of VEGF-A, VEGFR1, and VEGFR2 in pregnant women[31]. A 2021 study showed significantly higher protein and mRNA levels of VEGF and VEGFR-1 receptors in the placenta of physically active pregnant women than in their inactive counterparts[32]. Adipokines may play a pivotal role in obesity-related angiogenesis, with elevated leptin levels in obesity potentially enhancing angiogenesis via the upregulation of VEGFR expression and adhesion molecule expression. Therefore, it is plausible that increased secretion of angiogenic molecules in obesity could be mediated by increased adipokine expression. The increased serum VEGFR-1 levels in pregnant women with obesity compared with control pregnant women could be explained by the contribution of obesity to the release of VEGFR-1-mediated angiogenic molecules.

Study Limitations

Although this investigation sheds light on the interplay between LXA4 and inflammatory cytokines in maternal obesity, it is important to recognize several constraints in this study. The single-center approach limits population diversity, potentially restricting the applicability of the results to broader contexts. Despite the sample size being sufficient for the preliminary investigation of plasma LXA4 levels and inflammatory cytokines, its relatively small scale could weaken the statistical power and obscure specific potential effects. Replicating our findings using varied sample sizes and recruitment criteria can enhance their reliability. However, within our experimental framework, which is characterized by a moderate effect size, we achieved statistical significance and robust results. We should note that we only measured plasma LXA4 levels during the second trimester, thus missing the opportunity to monitor LXA4 fluctuations across different pregnancy stages. Nevertheless, this study has notable strengths. This study is the first to compare LXA4 levels in pregnant women with obesity with those in normal-weight pregnant women, offering valuable insights into the mechanisms driving obesity-related inflammation during pregnancy. The prospective cohort design permits real-time tracking of patient progress and identification of temporal relationships between variables. Consequently, this study not only contributes to existing scientific knowledge but also serves as a basis for future research. These future efforts could further clarify the pathophysiology of obesity-associated inflammation and potentially identify novel diagnostic markers and therapeutic interventions.

Conclusion

This study adds significantly to the body of scientific knowledge by demonstrating that increased BMI has potential effects on plasma lipoxin, cytokine, and angiogenesis-related factor levels during pregnancy. The precise mechanisms by which maternal obesity influences plasma LXA4, sFlt1, and cytokine levels remain incompletely understood. However, the observed differences between the two groups suggest a disruption of normal metabolic systems in women with obesity. Growing evidence suggests that these disruptions may significantly impact the body's normal changes during pregnancy and contribute to various health issues related to obesity. Consequently, changes in lipid balance during pregnancy may play a role in inadequate placental growth and compromised endothelial function. Given the growing global obesity epidemic, our findings highlight the urgent need for further investigation in this area. The physiological changes induced by obesity during pregnancy have both immediate and long-term health implications for the mother and developing fetus, and understanding these processes is critical to improving maternal and child health outcomes. By improving our understanding of these processes, we can better guide the development of targeted therapeutic strategies and preventive interventions, thereby ensuring healthier pregnancies and better health outcomes for both mothers and their offspring.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Inonu University Clinical Research Ethics Committee (approval number: 2021/114, date: 31.03.2021).

Informed Consent: The participants received comprehensive written and verbal details about the study, and their informed consent was duly obtained

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Comparison of maternal complications between fresh and frozen embryo transfer during gestation

Gebelikte taze ve dondurulmuş embriyo transferiyle ilişkili maternal komplikasyonların karşılaştırılması

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Abstract

Objective: Maternal complications in infertile women undergoing in vitro fertilization are an important discussion, and patients should be informed about these complications depending on the method of embryo transfer. In this study, maternal complications during gestation were compared between frozen and fresh embryo transfer in infertile women who underwent in vitro fertilization at Shariati Hospital from 2018 to 2021.

Materials and Methods: This study was a retrospective cohort study, and patient data were collected using archive files. From 396 in vitro fertilization patients, 302 were in the frozen embryo transfer group and 94 were in the fresh embryo transfer group. Patients in both groups were similar in terms of the number of transferred embryos and age (p>0.05). Data regarding threatened miscarriage, early miscarriage, placenta previa occurrence, gestational hypertension, preterm birth, gestational diabetes, and pre-eclampsia were gathered and compared between the two groups.

Results: The rates of threatened miscarriage, placenta previa, gestational hypertension, gestational diabetes, preterm birth, and pre-eclampsia were not significantly different between the fresh and frozen embryo transfer groups (p>0.05). However, the early miscarriage rate in the fresh embryo transfer group was significantly higher (34% vs. 16.2%, p<0.001).

Conclusion: According to the results of this study, maternal complications, except early miscarriage, were not different between fresh and frozen embryo transfer. However, frozen embryo transfer is safer in terms of the early miscarriage rate.

Keywords: Embryo transfer, threatened miscarriage, placenta previa, gestational diabetes, gestational hypertension

Öz

Amaç: İn vitro fertilizasyon uygulanan infertil kadınlarda maternal komplikasyonlar önemli bir tartışma konusu olup, embriyo transfer yöntemine bağlı olarak hastaların bu komplikasyonlar hakkında bilgilendirilmesi gerekmektedir. Bu çalışmada, 2018-2021 yılları arasında Shariati Hastanesi'nde in vitro fertilizasyon uygulanan infertil kadınlarda, dondurulmuş ve taze embriyo transferleriyle ilişkili maternal komplikasyonlar karşılaştırıldı.

Gereç ve Yöntemler: Bu çalışma retrospektif bir kohort çalışması olup hasta verileri arşiv dosyaları kullanılarak toplandı. Üç yüz doksan altı tüp bebek hastasının 302'si donmuş embriyo transfer grubunda, 94'ü ise taze embriyo transfer grubunda yer aldı. Transfer edilen embriyo sayısı ve yaş açısından her iki gruptaki hastalar benzerdi (p>0,05). Düşük tehdidi, erken düşük, plasenta previa oluşumu, gebelik hipertansiyonu, erken doğum, gebelik diyabeti ve preeklampsi ile ilgili veriler toplandı ve iki grup arasında karşılaştırma yapıldı.

Bulgular: Düşük tehdidi, plasenta previa, gestasyonel hipertansiyon, gestasyonel diyabet, erken doğum ve preeklampsi oranları taze ve dondurulmuş embriyo transfer grupları arasında anlamlı farklılık göstermedi (p>0,05). Ancak taze embriyo transfer grubunda erken düşük oranı anlamlı derecede yüksekti (%34 vs. %16,2, p<0,001).

Sonuç: Bu çalışmanın sonuçlarına göre, taze ve dondurulmuş embriyo transferleri arasında erken düşük dışında maternal komplikasyonlar açısından farklılık bulunmadı. Ancak donmuş embriyo transferi erken düşük oranı açısından daha güvenli idi.

Anahtar Kelimeler: Embriyo transferi, düşük tehdidi, plasenta previa, gebelik diyabeti, gebelik hipertansiyonu

PRECIS: The early miscarriage rate in fresh embryo transfer was higher, but other complications were not different between the two groups.

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Introduction

Infertility is defined as failure to achieve pregnancy after a year of unprotected sexual intercourse without the use of contraceptive methods. Infertility affects an average of 10% of couples of the reproductive age. Risk factors for infertility include old reproductive age, genetic disorders, infections, and environmental pollutants[1-3].

There are few methods of assisted reproductive technology, such as in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI), and intrauterine insemination. In IVF, eggs extracted from the ovary are fertilized with sperm in the laboratory. If infertility is also caused by a male factor, ICSI is also performed with IVF[1-3].

In IVF, there are two methods of embryo transfer: Frozen and fresh. In fresh embryo transfer, the embryos are transferred to the uterus in the same hormonal stimulation cycle. However, in frozen embryo transfer, the embryos are frozen and later taken to the uterus in a normal cycle or a cycle in which the endometrium has been prepared with hormone therapy. Factors affecting the selection of frozen or fresh embryos can depend on the progesterone level of the mother, endometrial thickness, polycystic ovary syndrome, and maternal age[1-3].

Many studies have compared fresh and frozen embryo transfer in terms of pregnancy outcomes and complications. However, their results vary and do not provide a firm conclusion on the matter. The goal of this study was to compare maternal complications in two methods of IVF pregnancies, frozen and fresh embryo transfer, during pregnancy.

Materials and Methods

This is a retrospective cohort study and was approved by the Ethics Committee of Tehran University of Medical Sciences (approval number: IR.TUMS.MEDICINE.REC.1400.061, date: 17.04.2021). All data were gathered through patient files in the hospital and phone calls. Patient information was kept private, and each patient was given a specific code to keep the information private. All stages of this study complied with the Declaration of Helsinki.

Study samples were infertile women treated with IVF/ICSI who were referred to Shariati Hospital Infertility Center from 2018 to 2021 and became pregnant. The exclusion criteria were unable to make phone calls, lack of data files, if embryo or egg was donated, and if pregnancy was surrogacy.

Three hundred ninety-six infertile women received IVF/ICSI treatment. Three hundred two of them (76%) received frozen embryo transfer and 94 of them (24%) received fresh embryo transfer. Patients in both groups were similar in terms of age (shown in Figure 1) and number of transferred embryos ($p>0.05$). Endometrial preparation for the frozen embryo transfer group was performed artificially using hormones. Data concerning maternal complications were gathered, and the two groups of fresh and frozen embryo transfer were compared in terms of threatened miscarriage, early miscarriage, placenta previa occurrence, gestational hypertension, gestational diabetes, preterm birth, and pre-eclampsia.

The two groups were not similar in size due to the lack of fresh embryo transfer patients who were referred to the hospital for treatment. Frozen embryo transfer was much more popular; therefore, embryo transfers are mostly frozen. This matter should not significantly affect the statistical outcomes of this study because of the high patient count in both groups compared to past studies[4].

Statistical Analysis

Data regarding infertility duration, number of oocytes retrieved, total gonadotrophin dose used, and number of blastocysts did not exist in the hospital files or were not gathered; therefore, they are not mentioned in this study. In addition, the pre-implantation genetic testing for aneuploidy) test was not performed for any patient.

Comparison of the groups was performed using Fisher's exact test, independent sample t-test, and chi-square test. Data analysis was performed using IBM SPSS 26 and significance level was less than 0.05.

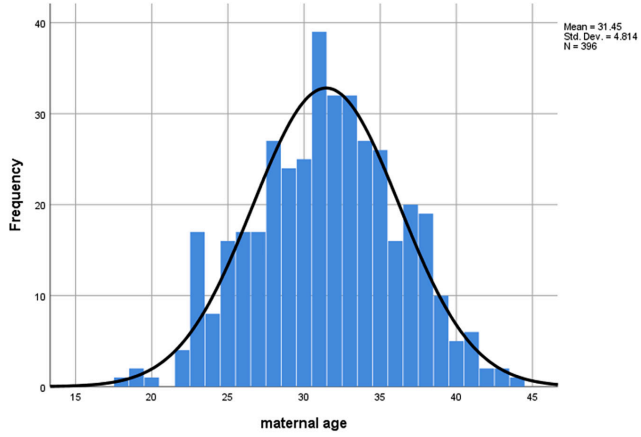

Figure 1. Maternal age in all patients

Threatened miscarriage: Vaginal bleeding in the first trimester without fetal loss while the cervix is closed.

Early miscarriage: Miscarriage before the 13th week of pregnancy.

Placenta previa: The placenta or part of it is placed on the cervical outlet.

Gestational diabetes: Glucose tolerance test disorder first diagnosed in the second or third trimester.

Gestational hypertension: Diastolic blood pressure greater than 90 mm Hg or systolic blood pressure greater than 140 mm Hg when diagnosed for the first time after the 20th week of pregnancy and not accompanied by proteinuria or end organ damage.

Pre-eclampsia: Diastolic blood pressure greater than 90 mm Hg or systolic blood pressure greater than 140 mm Hg when diagnosed for the first time after the 20th week of pregnancy and accompanied by proteinuria or end organ damage.

Results

The patient characteristics in both groups are shown in Table 1. Patient's age was 31.4 vs. 31.6 years and was similar in both groups (p=0.722). The number of transferred embryos was 2.7 vs. 2.5 and was similar between groups (p=0.081).

Maternal complications are listed in Table 2. Early miscarriage in the frozen vs. fresh group was 16.2% vs. 34%, and the fresh embryo transfer group was significantly higher than the frozen embryo transfer group (p<0.001). Threatened miscarriage was 9.9% vs. 7.4% and was not different between the two groups (p=0.469). Placenta previa occurrence was 4.3% vs. 4.8% and was not different between the two groups (p=0.743). Preterm birth was 26.8% vs. 24.5% and was not different between the two groups (p=0.651). Gestational diabetes was 16.9% vs. 15.3% and was not different between the two groups (p=0.098). Gestational hypertension was 8.2% vs. 6.7% and was not different between the two groups (p=1). Pre-eclampsia was 5.3% vs. 3.3% and was not different between the two groups (p=0.744).

It is worth mentioning that other maternal complications such as premature rupture of the membrane did not occur in any patient.

Table 1. Characteristics of IVF patients in two groups of frozen and fresh embryo transfer

	Frozen embryo transfer (302 patients)		Fresh embryo transfer (94 patients)		
	Mean	SD	Mean	SD	p-value
Female age (year)	31.4	4.7	31.6	4.9	0.722
Number of transferred embryos	2.7	0.5	2.5	0.6	0.081

IVF: In vitro fertilization, SD: Standard deviation

Discussion

Early Miscarriage

In past studies, the early pregnancy loss rate in frozen vs. fresh embryo transfer was not significantly different[4-7] and is as follows: 7.8% vs. 6.8%[4], 13.3% vs. 19.4%[5], 9.6% vs. 13.1%[6] and 21.5% vs. 25.3%[7]. Zargar et al.[8] found that first trimester loss was much higher in the fresh embryo transfer group (17.69% vs. 23.01%). Our results (16.2% vs. 34%) show that the chance of early miscarriage in the fresh embryo transfer group is 2.66 times higher than that in the frozen embryo transfer group. Higher early miscarriage rate in fresh embryo transfer may be due to the effect of hormones on the endometrium. Because in fresh embryo transfer, the embryos are taken to the uterus in the same ovarian stimulation cycle, the endometrium is under the influence of high levels of progesterone and estrogen. Theories have proposed that estrogen interferes with the angiogenesis of the endometrium and therefore has a negative effect on the continuation of pregnancy. In addition, ovulation stimulation by hormone therapy causes incoordination between the endometrium and embryo and thus has a negative effect on the implantation of the embryo in the uterus[5,9,10].

Threatened Miscarriage

Korosec et al.[11] found that first trimester bleeding occurrence was not different between the frozen and fresh groups (6.6% vs. 8.1%). Our results show no difference between groups and are similar to those of a previous study (9.9% vs. 7.4%).

Placenta Previa

Wikland et al.[12] concluded that placenta previa occurrence is not different between frozen and fresh embryo transfer (1% vs. 2.5%). However, according to Korosec et al.[11], Sazonova et al.[13] and Sha et al.[14], placenta previa occurrence was much higher in fresh embryo transfer [adjusted odds ratio (AOR) 0.32 [5% confidence interval (CI) 0.19-0.55)[11] and 0% vs. 3.5%[13]]. Ishihara et al.[15] found that placenta previa occurrence was much higher in frozen embryo transfer (AOR

Table 2. Maternal complications of IVF patients in two groups of frozen and fresh embryo transfer

	Frozen embryo transfer (302 patients)		Fresh embryo transfer (94 patients)		
	quantity	percentage	quantity	percentage	p-value
Threatened miscarriage	30	9.9%	7	7.4%	0.469
Early miscarriage	49	16.2%	32	34%	<0.001
Placenta previa occurrence	11	4.3%	3	4.8%	0.743
Preterm birth	81	26.8%	23	24.5%	0.651
Gestational diabetes	41	16.9%	9	15.3%	0.098
Gestational hypertension	20	8.2%	4	6.7%	1
Pre-eclampsia	13	5.3%	2	3.3%	0.744

IVF: In vitro fertilization

3.16, 95% CI 1.71-6.23). Also, Rombauts et al.[16] concluded that increased risk of placenta previa is not related to the type of embryo transfer (fresh and frozen), but is related to the type of endometrial preparation. Our results showed no difference between frozen and fresh embryo transfer (4.3% vs. 4.8%).

Preterm Birth

Preterm birth rate was not different in frozen vs. fresh embryo transfer according to past studies[6,11,17-19] (18.7% vs. 15.9%[6], 9% vs. 12.1%[11], 21.2% vs. 18.8%[17], 4.9% vs. 5.8%[18], and 7.2% vs. 7.5%[19]). However, some studies found that the preterm birth rate was higher in fresh embryo transfer[7,8,15] [0% vs. 11.1%[7], 3.93% vs. 8.3%[8] and AOR 0.90 (95% CI 0.82-0.98)[15]]. Our results showed no difference between frozen and fresh embryo transfer (26.8% vs. 24.5%).

Gestational Diabetes

Past studies have shown that gestational diabetes is not different between frozen and fresh embryo transfer[4,7,8,12-14,18,19] (3.1% vs. 3.9%[4], 1.8% vs. 0%[7], 23.66% vs. 24.64%[8], 2.9% vs. 2%[12], 0.9% vs. 1.5%[13], 1.3% vs. 1.8%[18] and 7.2% vs. 8%[19]). Our results show no difference between groups and are similar to those of past studies (16.9% vs. 15.3%).

Gestational Hypertension

Many studies have found that gestational hypertension is not different between frozen and fresh embryo transfer[4,6,7,12,18] (0.9% vs. 1.1%[4], 2.3% vs. 1.2%[6], 3.5% vs. 3.6%[7], 1.9% vs. 1%[12] and 0.8% vs. 1.3%[18]). However, another study by Ishihara et al.[15] found that gestational hypertension was higher in frozen embryo transfer [AOR 1.58 (95% CI 1.35-1.86]). Our study showed no difference between the frozen and fresh groups (8.2% vs. 6.7%).

Pre-eclampsia

Some studies have shown that pre-eclampsia is not different between frozen and fresh embryo transfer[4,7,8,12,18,19] (4.4% vs. 3.3%[4], 7.0% vs. 7.3%[7], 7.3% vs. 7.66%[8], 2.9% vs. 3%[12], 0.5% vs. 0.3%[18] and 2% vs. 1.4%[19]). However, other studies found a higher pre-eclampsia rate in frozen embryo transfer[6,13,20] [4.4% vs. 1.4%[6], AOR 1.32 (95% CI 1.07-1.63) [13] and 3.1% vs. 1%[20]]. Our study found no difference between the frozen and fresh groups (5.3% vs. 3.3%).

In fresh embryo transfer, the time to get pregnant is shorter compared with frozen embryo transfer. However, frozen embryo transfer has some advantages, such as reducing the risk of contracting OHSS, the ability to characterize embryo quality through genetics before implantation, and adjusting the natural physiological environment for better implantation.

In general, the early miscarriage rate, gestational hypertension rate, pre-eclampsia rate, and placenta previa rate for fresh and frozen embryo transfer differ in past studies. However, all studies concluded that the gestational diabetes rate and threatened miscarriage rate are similar in fresh and frozen embryo transfer.

Study Limitations

The retrospective nature of this study was a limitation. Prospective cohort studies and accurate long-term follow-up of patients can lead to a better understanding of the complications of embryo transfer methods. For future studies, it is suggested to evaluate the cost-effectiveness of these methods.

Conclusion

Fresh embryo transfer can increase the early miscarriage rate in IVF patients, and frozen embryo transfer is safer.

Ethics

Ethics Committee Approval: This is a retrospective cohort study and was approved by the Ethics Committee of Tehran University of Medical Sciences (approval number: IR.TUMS.MEDICINE.REC.1400.061, date: 17.04.2021).

Informed Consent: Informed consent was obtained from all participants.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.H., Concept: S.H., M.Z., S.M.G., Design: S.H., M.Z., H.Z., Data Collection or Processing: S.H., M.Z., S.M.G., Analysis or Interpretation: S.H., H.Z., Literature Search: M.Z., S.M.G., Writing: S.H., M.Z., S.M.G.

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Does the use of low-molecular-weight heparin during pregnancy change the expression of PD-1 and PDL-1 in women with recurrent pregnancy loss?

Tekrarlayan gebelik kaybı olan kadınlarda gebelikte düşük moleküler ağırlıklı heparin kullanımı PD-1 ve PDL-1 ekspresyonunu değiştirir mi?

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Abstract

Objective: The programed cell death gene-1 ligand (PDL-1) is expressed by villous syncytiotrophoblasts, cytotrophoblasts, and fetal cells in close contact with maternal tissue and blood. Programmed cell death gene-1 (PD-1) and the PDL-1 pathway cooperate with human leucocyte antigen-G (HLA-G), expressing intermediate trophoblastic cells and syncytiotrophoblasts to inhibit the function of activated T-cells. With this mechanism, the immunosuppressive microenvironment protects the placenta. This study investigated changes in *PD-1* and *PD-L1* gene expression in patients with a history of recurrent pregnancy loss (RPL).

Materials and Methods: Sixty patients participated in the study and were divided into three groups. Group 1 (G1): healthy pregnancy, G2: RPL but not low-molecular-weight heparin (LMWH), and G3: RPL and *LMWH. PD-1* gene expression in placental tissue samples was measured by reverse-transcriptase polymerase chain reaction and PD-L1 Elisa assay, and the study was supported by histopathology.

Results: The PD-L1 value decreased significantly in G2. A significant difference was observed between the groups in *PD-1* gene expression levels in G1 and G2. It was observed that vascularization increased and the villi structures intensified in G3. In G2, there was villus dysplasia in the placenta, enlargement in the intervillous region, and fibrin deposition. It was observed that the villi structures in G3 returned to a morphology similar to that of G1.

Conclusion: T-cells are activated in patients using LMWH, and a new therapeutic strategy can be developed to prevent pregnancy loss by targeting the PD-1 pathway.

Keywords: Low molecular weight heparin, placental disorders, pregnancy complications, recurrent pregnancy loss

Öz

Amaç: Programlanmış hücre ölüm geni ligandı-1 (PDL-1), maternal doku ve anne kanı ile yakın temas halinde olan villöz sinsityotrofoblastlar, sitotrofoblastlar ve fetal hücreler tarafından eksprese edilir. Programlanmış hücre ölüm geni-1 (PD-1) ve PDL-1 yolu, etkinleştirilmiş T hücrelerinin işlevini inhibe etmek için ara trofoblastik hücreleri ve sinsityotrofoblastları eksprese eden insan lökosit antijeni-G (HLA-G) ile işbirliği yapar. Bu mekanizma ile immünosüpresif mikroçevre plasentayı korur. Bu çalışmada tekrarlayan gebelik kaybı (RPL) öyküsü olan hastalarda *PD-1* ve *PD-L1* gen ekspresyonlarındaki değişikliklerin araştırılması amaçlandı.

PRECIS: T-cells are activated in patients using LMWH, and a new treatment strategy can be developed to prevent pregnancy loss by targeting the PD-1 pathway.

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Gereç ve Yöntemler: Çalışmaya 60 hasta katıldı; üç gruba ayrıldı. Grup 1 (G1): sağlıklı gebelik, G2: RPL ama düşük moleküler ağırlıklı heparin (DMAH) kullanmıyor ve G3: RPL ve DMAH kullanıyor. Plasental doku örneklerinde PD-1 gen ekspresyonu revers-transkriptaz polimeraz zincir reaksiyonu, PD-L1 Elisa testi ile ölçüldü ve çalışma histopatoloji ile desteklendi.

Bulgular: PD-L1 değeri G2'de anlamlı olarak azaldı. G1 ve G2'deki *PD-1* gen ekspresyon düzeylerinde gruplar arasında anlamlı bir fark gözlendi. G3'te vaskülarizasyonun arttığı ve villus yapılarının yoğunlaştığı gözlendi. G2'de plasentada villus displazisi, intervillöz bölgede genişleme ve fibrin birikimi mevcuttu. G3'teki villus yapılarının G1'e benzer bir morfolojiye döndüğü gözlendi.

Sonuç: DMAH kullanan hastalarda T-hücreleri aktive olur ve PD-1 yolağını hedefleyerek gebelik kayıplarını önlemek için yeni bir terapötik strateji geliştirilebilir.

Anahtar Kelimeler: Düşük moleküler ağırlıklı heparin, plasental bozukluklar, gebelik komplikasyonları, tekrarlayan gebelik kayıpları

Introduction

Human pregnancy is considered to be a unique immunological paradigm. In contrast, immunological events during pregnancy require maternal tolerance to the semi-allogeneic fetus and maintenance of a robust immune system to protect the mother and fetus against pathogens. Pregnancy has specific cellular and molecular mechanisms that regulate and enhance the immune environment[1]. Placental development, which begins in the early pregnancy period with the invasion of fetal trophoblast cells into the decidua, is significant for the continuation of pregnancy. The survival of the developing embryo and fetus requires establishing immune tolerance by inactivating the immune system on the maternal side, with the placenta thought to be provided by the trophoblast. The trophoblastic cells in the human placenta have different types, depending on their location, specific functions, and gene expression profiles[2].

Programmed cell death gene-1 ligand (PDL-1) is expressed by villous syncytiotrophoblasts, cytotrophoblasts, and fetal cells in close contact with maternal tissue and blood[3]. Programmed cell death gene-1 (PD-1) and the PDL-1 pathway cooperate with human leucocyte antigen-G (HLA-G), expressing intermediate trophoblastic cells and syncytiotrophoblasts to inhibit the function of activated T-cells. With this mechanism, the immunosuppressive microenvironment protects the placenta[2].

Recurrent pregnancy loss (RPL) is defined as before 20 weeks of pregnancy, the loss of three or more consecutive pregnancies[4]. Approximately 1-2% of all pregnant women are affected by RPL[5]. Accepted etiologies for RPL include antiphospholipid antibody syndrome (APAS), uncontrolled diabetes mellitus, parental chromosomal abnormalities, specific uterine anatomical abnormalities, and untreated hypothyroidism. Other possible etiologies include hereditary and/or acquired thrombophilias, additional endocrine disorders, environmental causes, and immunological abnormalities. After evaluation for these etiologies, more than 33% of all cases remain unexplained RPL[6]. Low-molecular-weight heparin (LMWH) has both anticoagulant and anti-inflammatory effects. It is widely used in the treatment of recurrent pregnancy loss when used alone or in combination with other agents such as acetylsalicylic acid[7-9]. In many studies, it has been determined that LMWH shows positive results in live births and reduces complications such as preeclampsia and preterm birth[10-12]. However, the mechanisms by which LMWH may be effective during pregnancy remain unclear.

This study investigated the changes in *PD-1* and *PD-L1* gene expressions in patients with recurrent miscarriages using LMWH.

Materials and Methods

This study was reviewed and approved by the Human Research Ethics Committee (Sivas Cumhuriyet University - approval number: 2023-05/06, date: 23.05.2023). Informed consent was obtained from all participants included in the study.

This study accepted it as α=0.05, β=0.20, (1-β)=0.80. The power of the test was calculated as p=0,80248. Sixty patients participated in the study. They were divided into three groups: G1- healthy pregnancy (n=20), G2-RPL but not administered LMWH (n=20), and G3-RPL and administered LMWH (n=20). Placental tissues retrieved at birth were sampled using a random sampling method [13,14]. All patient groups were selected from singleton pregnancies and patients who had a live birth in the last trimester of their pregnancy.

Patients with APAS, uterine anomalies, genetic diseases, preeclampsia, kidney and liver disease, maternal diabetes, and intrauterine growth restriction were excluded from the study.

ELISA

Placental tissue samples taken from patients during delivery were preserved at -80 °C. The Human PD-L1 Elisa Kit (Cat. No: E3680Hu) from the Bioassay Technology Laboratory was used. The standard range of the kit was 20-7000 ng/L.

RT-PCR

Total RNA was isolated from tissues (GeneAll, Cat no:106-101). A Nanodrop spectrophotometer (Thermo Scientific, USA) was used to measure RNA concentrations. Following its catalog, HyperScript (GeneAll Cat # 601-710) was used to convert RNA samples to cDNA.

PD-1 and GAPDH (housekeeping gene) were investigated as molecular probes (qubit ssDNA Assay Kit and Life Technologies) using Sybr Green Mastermix (High Rox Dye) (Cat no: 801-051). All parameters are listed in Table 1. The 2- ΔΔCT method was used for data analysis, and the housekeeping gene was used Glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

Histological Analyses

Immediately after birth, the cord and placental membranes were carefully cleaned and fixed in 10% neutral buffered formalin for 24 h. Unbiased tissue sampling for each placenta was

performed using a uniform random sampling protocol[13,14]. The removed tissues were blocked by passing through increasing ethyl alcohol levels, xylol, and warm paraffin as per the routine histological tissue follow-up procedure. 3 μm sections were prepared from the blocked tissues, deparaffinized, and stained with hematoxylin-eosin dye. The morphological structures of primary, secondary, and tertiary villi, their vascular structures, and intervillous areas were evaluated in stained tissue sections. In addition, fibrin structure and syncytial trophoblast deposits on the peripheral surface of the villi were also included in the assessment[15].

Statistical Analysis

One way was the ANOVA test. All statistical analyses were performed using SPSS 22.0. Significant difference was set at $p<0.05$.

Results

PD-1 and PD-L1 ligands were studied in tissues taken from the placenta. The PD-L1 ligand was studied in tissue using the ELISA method, as shown in Figure 1.

In the study, the PD-L1 value was found in G1: 106.68±1.68 ng/mL; in G2: 93.51±2.33 ng/mL, and in G3: 103.54±0.51 ng/mL. It was observed that PD-L1 decreased significantly in G2 compared with G1 and G3 ($p<0.05$).

qRT-PCR was used to determine the expression levels of PD-1 in placental tissues, as shown in Figure 2.

When *PD-1* gene expression levels were examined, it was found to be 3.05 3.05±0.12 in G1, 1.82±0.23 in G2, and 2.82±0.84 in G3, while the control group was accepted as 1.00. There was a significant increase in the other groups compared with the PCR control group. While a significant difference was observed between the groups in G1 and G2, no significant difference was observed between G1 and G3, G2 and G3 ($p<0.05$).

After the histological evaluation in our study, an insufficient spiral artery in G2 decrease in villus volume and surface area was observed. On the other hand, in G3, the vessels are increased, and villous structures are seen in a concentrated state. There was villus dysplasia in G2 of the villi in the placenta, enlargement in the intervillous area, and fibrin deposition. An increase was also observed in the syncytial cell node located at the periphery of the villi in G2. In G3, it was observed that the villi structures returned to a morphology similar to that of the control group (Figure 3). The morphological structure of the villi on decidua was compared. Typical appearance of the villi

Table 1. Primers used

	5' Sequence 3'
PDCD1-F	ACAGTTTCCCTTCCGCTCAC
PDCD1-R	CAGTTTAGCACGAAGCTCTCC
GAPDH-F	ACGGATTTGGTCGTATTGGG
GAPDH-R	TGATTTTGGAGGGATCTCGC

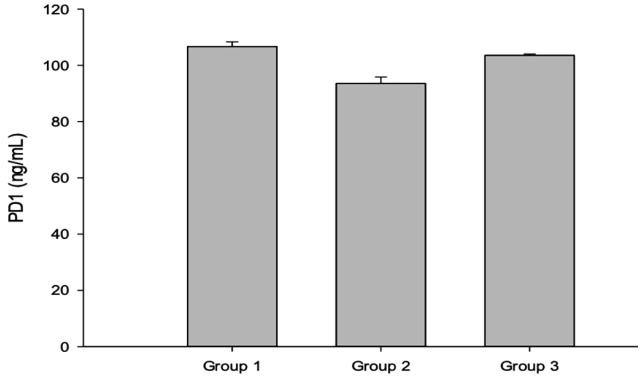

Figure 1. PDL-1 (ng/mL) value

PDL-1: Programed cell death gene-1 ligand

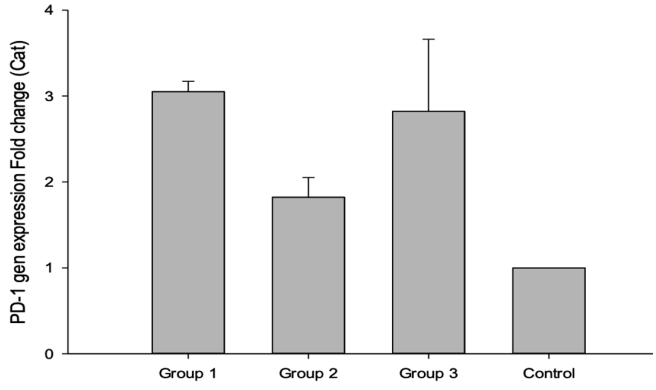

Figure 2. Change in *PD1* gene expression levels

PD1: Programmed cell death gene-1

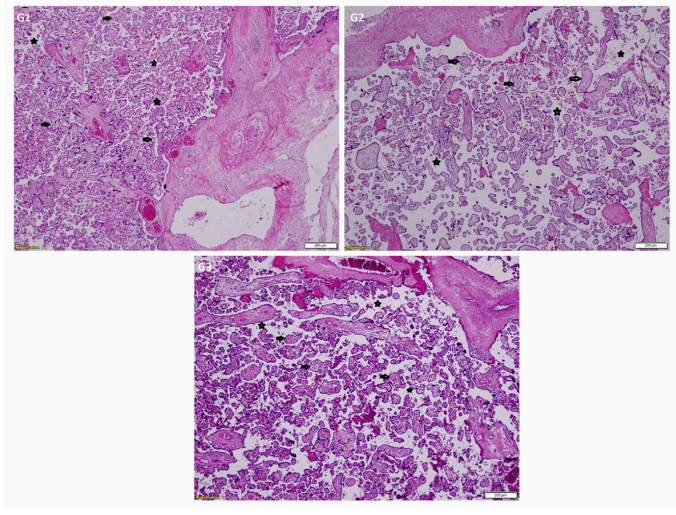

Figure 3. Comparison of the morphological structure of the villi on the decidua between the G1, G2, G3 groups of the histological changes in villous structures (arrow) and intervillous areas (star) (H&E staining, 10X Magnification)

H&E: Hematoxylin-eosin

and their arteries, villous structures with increased vascularity and blood supply, and decreased blood vessels in the separated villi were observed (Figure 4).

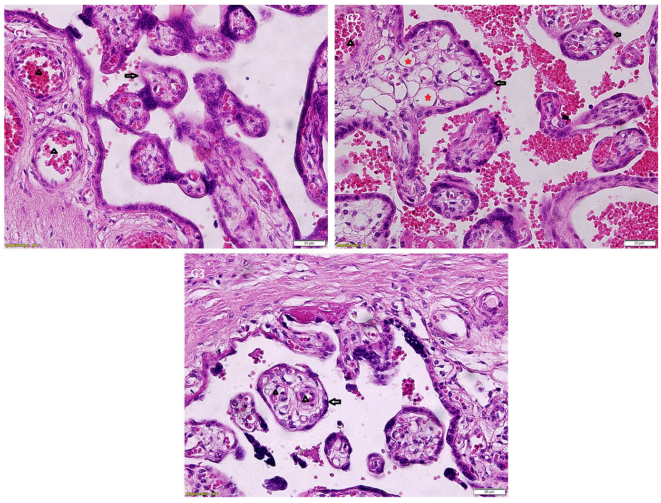

Figure 4. Comparison of structures of villi (arrow), villous arteries (triangle) and foam-like cells (red star) located in villi on the decidua between G1, G2, G3 groups (H&E staining, 40X Magnification)

H&E: Hematoxylin-eosin

Discussion

RPL is one of the most researched areas in medicine. The management of unexplained miscarriages is also challenging. Evaluation of placental morphology in the investigation of placental diseases is essential for understanding the pathogenesis of these diseases. This process involves the differentiation, migration, and division of a large number of cells and creates extensive vascularization[13].

In patients with a poor obstetric history, the area of the placental villi changes depending on the level of placental ischemia. The villous space increases with the invasion of syncytial trophoblasts, accumulation of fibrous tissue, and inadequate development of villus structures. Decreased surface area, volume, and vascularization of terminal and intermediate villi mediate maternal-fetal exchange. Perivillous fibrin deposition is characterized by the clustering of eosinophilic fibrin around the villi, and fibrin deposition impairs oxygenation around the villi, which also affects the morphology of the villi[13,15,16].

In our study, in patients with RPL who received LMWH treatment (G3), placental histopathological results showed a significant change in villi shrinkage, enlargement in the intervillous space, and fibrin deposition in the intervillous area compared with patients with RPL who did not receive LMWH (G2). However, there was no significant difference between the healthy pregnant group (G1). In the study of Ozdemir et al.,[13] it was stated that the changes in the structure of the villi and the intervillous area in the placentas of patients using LMWH were similar to our findings. However, there was no significant difference in the statistical comparison with the healthy pregnant group[13].

Deficits in maternal arterial remodeling are associated with the pathophysiology of major obstetric syndromes, including growth restriction. First, the rate at which maternal blood enters the placental intervillous space is adversely affected. Second, the involvement of the vascular smooth muscle causes a more intermittent perfusion of the placenta at the junctional site. Third, inadequate remodeling predisposes spiral arteries to acute atherotic changes. In our study, while there was foam-like structure accumulation in the spiral arteries in the decidua in the group (G2) that did not use LMWH, these structures were not observed in the healthy group (G1) and the group using LMWH (G3) (Figure 3).

Recent studies focusing on the use of LMWH have suggested insufficient evidence to apply this therapy in patients with unexplained recurrent miscarriages[17]. In Ozdemir et al.,[13] LMWH did not significantly affect the placental structure of cases with a history of recurrent miscarriage. In another study, 50 of the patients with RPL received aspirin therapy and 54 received LMWH; placental Doppler flow was similar between the groups, and the live birth rates of the groups were also similar[18].

In Rodger's meta-analysis, no effect of LMWH in reducing early pregnancy loss was observed in patients with prior placental pregnancy complications. In comparison, a statistically non-significant reduction in late pregnancy loss was observed[19]. In another study, Lu et al.[20] administered aspirin to groups of patients with miscarriage and abnormal prenatal platelet aggregation. LMWH was used in patients with high D-dimer levels, and it was observed that platelet aggregation and D-dimer levels decreased during pregnancy. An 89.2% live birth rate was reported in the group with unexplained recurrent miscarriage.

PD-1 is a molecule discovered by Freeman et al.[21] in 1992. Pdcd1 encodes PD-1 on the second chromosome. The programed cell death pathway is activated during the late stages of inflammation. In this pathway, T-cells become anergic by interacting with PD-1 on the surface of activated T-cells and its ligands, PD-L1 (B7-H1) and PD-L2 (B7-DC) [23]. PD-1 is expressed only on activated T lymphocytes. It has also been shown that PD-1 is expressed in B lymphocytes. It is believed to have a broader spectrum than CTLA-4 in immune regulation with this feature[23]. The physiological role of PD-1 is to maintain T-cell homeostasis and balance T-cell proliferation and activation. The binding of PD-1 expressed on the surface of activated T-cells to the PD-L1 ligand generates an inhibitory signal and decreases cytokine release[24].

The PD-1/PD-L1 signaling pathway is also an adverse costimulatory pathway. PD-1 is mainly expressed on the surface of activated T-cells, whereas PD-L1 is primarily expressed on antigen-presenting cells and immunologically immune regions (such as the placenta). It has been shown that PD-L1 expression

is abundant in the placenta[25,26]. Studies have reported that PD-L1 deficiency is associated with increased fetal resorption frequency and decreased fetal survival. The interaction between PD-1 and its ligand PD-L1 plays a critical role in establishing maternal- fetal tolerance and maintaining pregnancy by regulating T-cells[27]. An important inhibitory pathway that induces T-cell anergy is PD-1/PD-L1[28]. Studies of D'addio's model of PD-L1 blockade reported that PD-L1 blockade could reduce embryo size and embryo loss in model mice while increasing Th17 and Th1 cells in peripheral lymphoid tissues[29]. However, it has been determined that the absence or decrease in PD-L1 may cause spontaneous abortion pathogenesis[26]. In our study, an increase in *PD-1* gene expression level and PD-L1 amount was observed in patients administered LMWH compared with the group that did not.

This study is essential for investigating the effects of LMWH administration, a costly and complicated treatment, on *PD-1* gene expression and placental morphology in patients with unexplained recurrent miscarriages. In conclusion, LMWH may be a promising treatment for placenta-mediated pregnancy complications, particularly for recurrent pregnancy loss, severe preeclampsia, placental abruption, and small gestational age complications. More extensive population studies are needed to better understand this issue.

Conclusion

In line with these results, it was concluded that T cells are activated in patients using LMWH, and a new therapeutic strategy can be developed to prevent pregnancy loss by targeting the PD-1 pathway.

Ethics

Ethics Committee Approval: This study was reviewed and approved by the Human Research Ethics Committee (Sivas Cumhuriyet University - approval number: 2023-05/06, date: 23.05.2023).

Informed Consent: Informed consent was obtained from all participants included in the study.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: B.K., İ.K., Concept: B.K., C.H., Z.D.Ş.İ., Design: B.K., C.H., Data Collection or Processing: B.K., C.H., Z.D.Ş.İ., İ.K., Analysis or Interpretation: B.K., C.H., Z.D.Ş.İ., Literature Search: B.K., C.H., Z.D.Ş.İ., Writing: B.K., C.H., Z.D.Ş.İ., İ.K.

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

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Integrated analysis of differentially expressed genes implicated in ovarian cancer progression

Diferansiyel olarak ifade edilen genlerin entegre analizi yumurtalık kanserinin ilerlemesinde rol oynar

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Abstract

Objective: Ovarian cancer (OC) is a common gynecological malignancy associated with high morbidity and generally poor prognosis despite treatment. The aim of this study was to understand the influence of gene expression differences and pathways in OC development and progression.

Materials and Methods: One hundred and thirty-three OC samples and 34 normal ovarian tissues were included in the study from the Gene Expression Omnibus database. GeneSpring Software was used to obtain differentially expressed genes (DEGs) in all stages comparing tumor and normaltissue. DEGs were analyzed using the DAVID interface for Kyoto Encyclopedia of Genes and Genomes pathway analysis. Most most connected genes were selected as hub genes for each stage using the STRING application in Cytoscape software.

Results: DEGs were found to be associated with cell cycle and herpes simplex virus infection pathways. A total of 19 genes (*ACTB, AKT1, ALB, CTNNB1, EGFR, EP300, ESR1, FN1, GAPDH, HSPA4, IL6, JUN, MYC, PTEN, RPS27A, SRC, TNF, TP53* and *UBC*) were identified as hub genes. Among the hub genes, the *TP53* gene was found to have the highest level of connections in all stages. EGFR, RPS27A, and AKT1 were found to have high numbers of connections in stages II, III, and IV, respectively.

Conclusion: The results of the current study may provide new insights into OC pathogenesis and suggest potential prognostic and therapeutic targets.

Keywords: Ovarian cancer, gene expression, hub genes, integrated analysis

Öz

Amaç: Yumurtalık kanseri, tedavi çabalarına rağmen yüksek morbiditeye sahip olup ve genellikle kötü prognoz ile ilişkili yaygın bir jinekolojik malignite olarak bilinmektedir. Çalışmamızda, gen ekspresyon farklılıklarının ve moleküler yolakların yumurtalık kanseri gelişimi ve ilerlemesindeki rolünün araştırılması amaçlanmıştır.

Gereç ve Yöntemler: Çalışmaya yüz otuz üç yumurtalık kanseri ve 34 normal yumurtalık dokusu örneği Gene ExpressionOmnibus veri tabanından indirilerek dahil edilmiştir. Tüm evrelerde diferansiyel olarak ifade edilen genleri (DEG'ler) elde etmek için GeneSpring Yazılımı tümör ve normali karşılaştırarak kullanıldı. DEG'ler, Kyoto Genler ve Genomlar Ansiklopedisi yolak analizi için DAVID arayüzü kullanılarak analiz edilmiştir. Her evredeki merkezi genler, Cytoscape yazılımındaki STRING uygulaması kullanılarak en fazla bağlantıya sahip 15 gen şeklinde belirlenmiştir.

Bulgular: Diferansiyel olarak ifade edilen genler, hücre döngüsü ve herpes simpleks virüs enfeksiyonu yolakları ile ilişkili bulunmuştur. Toplam 19 gen (*ACTB, AKT1, ALB, CTNNB1, EGFR, EP300, ESR1, FN1, GAPDH, HSPA4, IL6, JUN, MYC, PTEN, RPS27A, SRC, TNF, TP53* ve *UBC*) merkezi genler olarak saptanmıştır. Merkezi genler arasında *TP53* geninin tüm evrelerde en yüksek düzeyde bağlantıya sahip olduğu bulunmuştur. EGFR, RPS27A ve AKT1'in sırasıyla evre II, evre III ve evre IV'te yüksek sayıda bağlantıya sahip olduğu dikkati çekmiştir.

Sonuç: Bu çalışmanın sonuçları over kanseri patogenezine ilişkin literatüre yeni bilgiler katabilir ve potansiyel prognostik ve terapötik hedefler önerebilir.

Anahtar Kelimeler: Yumurtalık kanseri, gen ekspresyonu, merkezi genler, entegre analiz

PRECIS: Using public gene expression microarray datasets, we have investigated differentially expressed genes and pathways playing a role in the ovarian cancer progression.

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Introduction

Ovarian cancer (OC) is a prevalent gynecological malignancy associated with high morbidity and generally poor prognosis. Although the 5-year survival rate for early-stage OC patients is 93%, the majority of patients (over 80%) are not diagnosed until the tumor progresses to stage III or IV[1]. Metastasis and recurrence (usually associated with increased chemoresistance) are frequent in ovarian cancer[2].

The poor prognosis and high mortality rate can be mainly attributed to the lack of early and effective detection methods. Thus, increased efforts are required to identify and comprehend new biomarkers and distinct targets of ovarian cancer. Illuminating genetic expression differences in ovarian cancers using the microarray method can be used for diagnostic, prognostic, or therapeutic purposes.

The aim of this study was to analyze gene expression differences in OC and to investigate the influence of associated genes and pathways on the development and/or progression of ovarian cancers using gene expression microarray datasets from stages I, II, III, and IV.

Materials and Methods

Gene Expression Microarray Data

The National Center for Biotechnology Information-Gene Expression Omnibus (NCBI-GEO) database is a free and publicly accessible database containing gene profiles. Gene expression profiles were selected from seven microarray datasets (GSE18520, GSE28044, GSE65986, GSE44104, GSE9891, GSE39204, and GSE63885) in the GEO database. The selected gene expression profiles were based on data obtained from human and normal ovarian tissues and the GPL570 platform (Affymetrix Human Genome U133 Plus 2.0 Array).

A total of 133 patient samples (Stage I: 47, Stage II: 21, Stage III: 41, Stage IV: 24) and 34 control samples were included in the study. From the GSE65986 dataset, 55 patients (Stage I: 30, Stage II: 5, Stage III: 11, Stage IV: 9), from the GSE44104 dataset, 60 patients (Stage I: 17, Stage II: 8, Stage III: 30, Stage IV: 5), from the GSE9891 dataset, 5 patients (Stage II), from the GSE39204 dataset, 3 patients (Stage II), from the GSE63885 dataset, 10 patients (Stage IV) were selected. Control samples were chosen from the GSE28044 dataset including 24 "non-malignant" tissue samples, and from the GSE18520 dataset including 10 "normal ovary" tissue samples. Gene expression microarray raw data for the samples described in the datasets were downloaded from the GEO database.

Identification of Differentially Expressed Genes (DEGs)

GeneSpring Software version 14.9_gx_pa was used to obtain DEGs between tumor and normal tissues. Although GeneSpring is not an open source software, it is user-friendly and has a useful interface for the analysis of genomic and omics data, offering multiple analysis and visualization results. During analysis, DEGs were defined using One-Way ANOVA statistical analysis between tumor and normal tissues, with a p-value threshold of <0.05 and a fold change of >2.0. The Benjamini-Hochberg correction method was used to reduce false positives.

Functional Enrichment Analysis of DEGs

In this study, the online tool DAVID (the Database for Annotation, Visualization and Integrated Discovery) was used to perform gene ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of DEGs. GO analysis included biological processes (BP), cellular components, and molecular functions (MF) categories. Pathway analysis is a functional analysis that matches genes to KEGG pathways. The cutoff criterion was set at p<0.05. A different pathway enrichment analysis tool, the g:GOSt (https://biit.cs.ut.ee/gprofiler/gost) embedded with the g:Profiler web server, was used to confirm the KEGG pathways identified for DEGs of each stage.

Construction of a Protein-protein Interaction (PPI) Network

Cytoscape software version 8.3.2, along with the search tool for the retrieval of interacting genes (STRING) application, was used to explore potential relationships between DEGs at different stages. Although Cytoscape requires knowledge for use, it is an open source software that produces networks containing more interactions compared with commercial software. According to the Cytoscape results, the top 15 genes with high connectivity were selected as hub genes based on PPI information. Hub genes were added to STRING, and GO and KEGG pathway analyses were conducted using DAVID to determine potential information. Another pathway enrichment analysis tool, g:Profiler g:GOSt (https://biit.cs.ut.ee/gprofiler/gost), was used to confirm the KEGG pathways identified for hub genes of each stage.

Analysis of Hub Gene Survival

Overall survival analysis was performed using the ene Expression Profiling Interactive Analysis) (http://gepia.cancer-pku.cn/detail.php), a web tool based on the Cancer Genome Atlas and Genotype-Tissue Expression) gene expression datasets[3]. Default settings, as the cut-off value set to median =50%, hazard ratio (HR) with 95% confidence intervals, and Logrank p-value were used in the single gene analysis module for every hub gene. HR information and log-rank p-values were displayed in the survival plots.

Results

Identifying DEGs

Genes that were expressed differently were defined by comparing the expression rate changes of samples taken from tumor tissues with those of normal tissues using a threshold value of >2.0 in GeneSpring. In stages I, II, III, and IV, 4836, 4249, 4702, and 4340 upregulated genes and 3830, 3421, 3533, and 3848 downregulated genes were identified, respectively.

Pathway Analysis of the DEGs

The most significant molecular pathways defined through pathway analysis of DEGs in tumor tissues using DAVID were the cell cycle for upregulated DEGs and herpes simplex virus infection for downregulated DEGs in stages I, II, III, and I. These pathways were confirmed using g:Profiler and g:GOS. Cell cycle was the most enriched pathway with p-values $5.469x10^{-13}$, $1.935x10^{-10}$, $8.087x10^{-14}$ and $9.804x10^{-11}$ in stages I, II, III, and IV, respectively, according to g:Profiler g:GOSt. Herpes simplex virus infection was the most enriched pathway with p-values $8.335x10^{-8}$, $1.547x10^{-7}$, $2.425x10^{-5}$ and $5.456x10^{-7}$ in stages I, II, III, and IV, respectively, according to g:Profiler g:GOSt. The top five enriched KEGG pathways for upregulated and downregulated DEGs of each stage according to DAVID are presented in Table 1.

GO Analysis of the DEGs

The BP, cellular components, and MF of overexpressed and underexpress genes were determined by GO analysis. Across all stages, upregulated DEGs were most closely associated with cell division in terms of BP, the cytosol in terms of cellular components, and protein binding in terms of molecular functions. Downregulated DEGs were closely related to cilium morphogenesis in terms of BP, cytoplasm in terms of cellular components, and metal ion binding in terms of MF Table 1. However, in stage IV, downregulated DEGs were more closely associated with the cellular component nucleus than with the cytoplasm.

PPI Network

Based on information obtained from publicly available databases such as STRING, PPI networks were constructed for DEGs in each class, and the top 15 genes with the highest level of connections were defined as hub genes Table 2.

KEGG and GO Analysis of Hub Genes

KEGG pathway analysis and GO analysis were performed for hub genes at each stage. According to KEGG pathway analysis using DAVID, hub genes were associated with Kaposi's sarcoma-associated herpesvirus infection in stages I and III and proteoglycans (PGs) in cancer in stages II and IV (Table 3). These pathways were confirmed using g:Profiler and g:GOS. According to pathway enrichment analysis using g:Profiler g:GOSt, Kaposi's sarcoma-associated herpesvirus infection pathway was enriched with a p-value $3.155x10^{-8}$ both in stages I and III. PGs in the cancer pathway were enriched with p-values $9.406x10^{-10}$ and $1.386x10^{-11}$ in stages II and IV, respectively, according to g:Profiler g:GOSt. GO analysis revealed that hub genes were generally associated with positive regulation of transcription DNA-templated in terms of BP, protein-containing complex in terms of cellular components, and enzyme binding in terms of MF Table 4.

Survival Analysis of Hub Genes

The overall survival analysis of 19 hub genes was performed using GEPIA with the default settings (the cut-off value set to median =50%, HRs with 95% confidence intervals). Considering the survival plots, HRs >1 were associated with worse overall survival. Among the hub genes, *MYC* (HR=1.3), epithelial growth factor receptor (*EGFR*) (HR=1.1), *EP300* (HR=1.1), *ESR1* (HR=1.1), *GAPDH* (HR=1.1), *IL6* (HR=1.1), *JUN* (HR=1.1), and *UBC* (HR=1.1) expression were found to be associated with worse overall survival for OC (Figure 1).

Discussion

Although early-stage OC exhibits a 5-year survival rate of approximately 93%, diagnosis is often delayed until Stage III or IV in over 80% of cases, contributing to its overall poor prognosis and high mortality. Therefore, the identification of new biomarkers is crucial for the early detection and development of novel treatment approaches. In alignment with the aim of this study, we obtained datasets from the GEO database to compare 133 OC samples with 34 normal tissue samples.

DEGs were analyzed for KEGG pathways using the DAVID Bioinformatics Database. Upregulated DEGs were primarily associated with the cell cycle, whereas downregulated DEGs were notably associated with herpes simplex virus 1 infection.

Dysregulation of the cell cycle is a hallmark of many cancers, including ovarian cancer. Control and timing of the cell cycle involve checkpoints and regulatory pathways that ensure the accuracy of DNA replication and chromosome segregation. These processes encompass candidate molecules for genetic variants that predispose patients to OC risk. Molecules crucial to the cell cycle, such as *CDK*, *CCNE*, and *E2F*, are overexpressed in various cancers, including ovarian cancer[4]. Studies conducted on OC samples reveal alterations in cell cycle phases, particularly in the G2 phase. Our findings, correlated with literature information, support the suggestion that cell cycle abnormalities in OC may be influenced by genetic variations in genes.

The most common manifestation of herpes simplex virus-1 infection is cold sores on the lips. However, some studies have indicated HSV-1 in various tumor cells. Recently identified herpes virus-associated growth factors with both transforming and transformation-suppressing activities are considered to be significant factors in tumor formation. Furthermore, in two cancer cases, serous ovarian carcinoma and certain prostate tumors, virus-encoded microRNAs were identified as potential cofactors in tumor formation[5]. Further research is needed to understand the mechanisms involved and potential therapeutic interventions.

In our study, 19 genes (*ACTB*, *AKT1*, *ALB*, *CTNNB1*, *EGFR*, *EP300*, *ESR1*, *FN1*, *GAPDH*, *HSPA4*, *IL6*, *JUN*, *MYC*, *PTEN*, *RPS27A*, *SRC*, *TNF*, *TP53*, and *UBC*) were identified as hub genes, with the top 15 genes having the most connections at

each stage. Among the hub genes, the *TP53* gene was found to have the highest level of connections in all stages. *EGFR*, *RPS27A,* and *AKT1* were found to have high numbers of connections in stages II, III, and IV, respectively.

The *TP53* protein is extensively studied and is best known as a DNA-binding transcription factor that can bind to hundreds of different promoter elements in the genome. This characteristic allows it to regulate the expression of numerous genes. Years

Table 1. KEGG pathway and gene ontology analysis of differentially expressed genes associated with ovarian cancer using DAVID

Stage I	Upregulated	**Pathways**	**Biological process**	**Cellular component**	**Molecular function**
		Cell cycle	Cell division	Cytosol	Protein binding
		Cellular senescence	Intracellular protein transport	Membrane	RNA binding
		Parkinson disease	Angiogenesis	Extracellular exosome	Cadherin binding
		Protein processing in the endoplasmic reticulum	Proteasome-mediated ubiquitin	Nucleoplasm	Identical protein binding
		p53 signaling pathway	Mitochondrial translation	Cytoplasm	Ubiquitin protein ligase binding
	Downregulated	Herpes simplex virus 1 infection	Cilium assembly	Cytoplasm	Metal ion binding
		AMPK signaling pathway	Regulation of transcription, DNA-templated	Nucleus	Protein binding
		Autophagy: Animal	Cilium movement	Nucleoplasm	Guanyl-nucleotide exchange factor activity
		FoxO signaling pathway	Cilia-dependent cell motility	Cytosol	Zinc ion binding
		Choline metabolism in cancer	Intracellular signal transduction	Axoneme	Protein serine/threonine kinase activity
Stage II	Upregulated	**Pathways**	**Biological process**	**Cellular component**	**Molecular function**
		Cell cycle	Cell division	Cytosol	Protein binding
		Epstein– Barr virus infection	Angiogenesis	Extracellular exosome	RNA binding
		Phagosome	Positive regulation of cell migration	Membrane	Cadherin binding
		p53 signaling pathway	Apoptotic process	Nucleoplasm	Identical protein binding
		Human T-cell leukemia virus 1 infection	Negative regulation of the apoptotic process	Cytoplasm	Ubiquitin protein ligase binding
	Downregulated	Herpes simplex virus 1 infection	Cilium assembly	Cytoplasm	Metal ion binding
		Autophagy: animal	Regulation of transcription, DNA-templated	Nucleus	Protein binding
		Choline metabolism in cancer	Cilium movement	Axoneme	ATP-dependent microtubule motor activity
		One carbon pool formed by folate	Regulation of transcription from RNA	Cytosol	Zinc ion binding
		SNARE interactions during vesicular transport	Cilia-dependent cell motility	Nucleoplasm	Protein serine/threonine kinase activity
		Pathways	Biological process	Cellular component	Molecular function
Stage III	Upregulated	**Cell cycle**	**Cell division**	**Cytosol**	**Protein binding**
		Prion disease	Angiogenesis	Nucleoplasm	RNA binding
		Parkinson disease	Protein catabolic process	Membrane	Cadherin binding
		Cellular senescence	Mitochondrial translation	Extracellular exosome	Identical protein binding
		Non-alcoholic fatty liver disease	Apoptotic process	Nucleus	Ubiquitin protein ligase binding

Table 1. Continued

Stage III	Downregulated	Herpes simplex virus 1 infection	Cilium assembly	Cytoplasm	Metal ion binding
		FoxO signaling pathway	Regulation of transcription, DNA-templated	Nucleus	Protein binding
		AMPK signaling pathway	Cilium movement	Cytosol	Zinc ion binding
		Autophagy: animal	Negative regulation of transcription	Axoneme	Guanyl-nucleotide exchange factor activity
		Choline metabolism in cancer	Intracellular signal transduction	Motile cilium	Protein serine/threonine kinase activity
		Pathways	**Biological process**	**Cellular component**	**Molecular function**
Stage IV	Upregulated	Cell cycle	Cell division	Cytosol	Protein binding
		Epstein– Barr virus infection	Angiogenesis	Membrane	Cadherin binding
		Human T-cell leukemia virus 1 infection	Positive regulation of transcription	Extracellular exosome	Identical protein binding
		Human papillomavirus infection	Apoptotic process	Nucleoplasm	RNA binding
		Cellular senescence	Cell migration	Cytoplasm	Ubiquitin protein ligase binding
	Downregulated	Herpes simplex virus 1 infection	Cilium assembly	Nucleus	Metal ion binding
		AMPK signaling pathway	Cilium movement	Cytoplasm	Protein binding
		FoxO signaling pathway	Regulation of transcription, DNA-templated	Cytosol	Zinc ion binding
		Autophagy: animal	Regulation of transcription from RNA	Axoneme	Guanyl-nucleotide exchange factor activity
		Antifolate resistance	Negative regulation of transcription	Nucleoplasm	ATP binding

of research on *TP53* have documented its fundamental role in controlling cell proliferation and regulating essential cellular processes that maintain genome integrity and stability[6]. The *TP53* protein responds to various stress signals such as DNA damage, hyperproliferative signals, hypoxia, oxidative stress, and ribonucleotide depletion. Upon activation, it primarily halts the cell cycle, triggers DNA repair, and initiates apoptosis. This leads to the suppression of cellular transformation and proliferation. Over the years, research has revealed TP53's involvement in other cellular processes such as metabolism, angiogenesis, immune responses, stem cell maintenance, and tumor-stromal cell crosstalk[7]. In all ovarian cancers, a significantly high mutation frequency ranging from 50% to 100% has been reported[8]. Moreover, studies have confirmed the overexpression of *TP53* in ovarian cancers, but its prognostic significance remains controversial[9-15]. In our study, the identification of *TP53* as the hub gene with the most connections across all stages and its detection as overexpressed are correlated with previous research findings.

The *EGFR* plays important roles in tumor initiation, angiogenesis, and metastasis[16]. Deregulation of *EGFR* has been reported in several malignancies as well as in ovarian cancer. EGFR expression has been detected in up to 90% of certain histotypes of ovarian tumors[17]. Previous investigations on OC have shown that the EGFR protein is overexpressed in 9-62% of cases and is associated with poor prognosis and decreased therapeutic responsiveness[18]. In patients with pancreatic tumors, specific histotypes of ovarian tumors, and lung cancer patients with EGFR mutations, EGFR inhibitors have been recommended as first-line treatment[16]. In our study, EGFR was found to have a high number of connections in stage II. We suggest that EGFR could be a potential biomarker for the diagnosis and prognosis of ovarian cancer.

Ribosomal protein S27A *(RPS27A)* encodes a ribosomal 40S subunit ribosomal protein. RPS27A is involved in ubiquitin production, regulating cell cycle progression, DNA repair, promoting proliferation, and inhibiting apoptosis. Furthermore, RPS27A is a direct transcriptional factor of p53 and is overexpressed in various organ malignancies such as kidney, breast, colon, lung, liver, brain, thymus, and cervix as well as in leukemia and is associated with poor prognosis[19]. RPS27A has been used as a prognostic biomarker in hepatocellular carcinoma and has been identified as a hub gene with increased expression in OC before[20]. In our study, *RPS27A* was found to be a hub gene in stage III cancer, suggesting the importance of RPS27A in tumorigenesis and OC.

Table 2. List of hub genes according to the stages

Stage I			Stage II			Stage III			Stage IV		
Gene	CD	EL	Gen	CD	EL	Gen	CD	EL	Gen	CD	EL
TP53	1070	↑	*TP53*	986	↑	*TP53*	1063	↑	*TP53*	1005	↑
ACTB	934	↑	*ACTB*	881	↑	*ACTB*	926	↑	*ACTB*	895	↑
GAPDH	874	↑	*GAPDH*	816	↑	*GAPDH*	868	↑	*AKT1*	862	↑
MYC	846	↑	*MYC*	778	↑	*MYC*	839	↑	*GAPDH*	824	↑
CTNNB1	829	↑	*CTNNB1*	755	↑	*CTNNB1*	800	↑	*CTNNB1*	797	↑
SRC	688	↑	*EGFR*	671	↑	*RPS27A*	706	↑	*MYC*	789	↑
UBC	632	↑	*SRC*	632	↑	*SRC*	663	↑	*EGFR*	708	↑
TNF	595	↓	*TNF*	561	↑	*TNF*	572	↑	*SRC*	655	↑
ALB	580	↓	*ALB*	538	↓	*PTEN*	562	↓	*TNF*	588	↑
PTEN	573	↓	*JUN*	513	↑	*ALB*	552	↓	*ALB*	556	↓
ESR1	554	↓	*FN1*	510	↑	*JUN*	546	↑	*PTEN*	544	↓
EP300	551	↓	*IL6*	510	↑	*HSPA4*	542	↑	*IL6*	529	↑
JUN	547	↑	*HSPA4*	506	↑	*EP300*	542	↓	*FN1*	523	↑
IL6	544	↑	*ESR1*	490	↓	*IL6*	531	↑	*EP300*	522	↓
FN1	536	↑	*EP300*	489	↓	*ESR1*	526	↓	*ESR1*	502	↓

CD: Connection degree, EL: Gene expression level, ↑: Up-regulated, ↓: Down-regulated

Table 3. KEGG pathway analysis of hub genes according to the stages using DAVID

	Pathways	p-value
Stage I	Kaposi's sarcoma-associated herpesvirus infection	8,90E-09
	Proteoglycans in cancer	1,30E-08
	Thyroid hormone signaling pathway	2,10E-08
	Hepatitis B	1,20E-07
	Pathways in cancer	4,90E-07
Stage II	Proteoglycans in cancer	2,70E-10
	Thyroid hormone signaling pathway	2,10E-08
	Hepatitis B	1,20E-07
	Kaposi's sarcoma-associated herpesvirus infection	3,50E-07
	Pathways in cancer	4,90E-07
Stage III	Kaposi's sarcoma-associated herpesvirus infection	8,90E-09
	Thyroid hormone signaling pathway	2,10E-08
	Hepatitis B	1,20E-07
	Proteoglycans in cancer	4,80E-07
	Human T-cell leukemia virus 1 infection	7,70E-07
Stage IV	Proteoglycans in cancer	4,30E-12
	Thyroid hormone signaling pathway	3,20E-10
	Pathways in cancer	2,10E-08
	Endometrial cancer	2,40E-08
	Human cytomegalovirus infection	2,50E-08

AKT1 is a member of the AKT serine/threonine protein kinase family that regulates various functions such as cell proliferation, survival, and metabolism. AKT is a key component of signaling pathways and is effective in both normal and malignant cells. *AKT1-3* are overexpressed in ovarian cancer. AKT activation is commonly observed in high-grade serous ovarian cancer[21]. It has been proposed that AKT1 is the main isoform responsible for OC cell proliferation and protection against apoptosis, playing a significant role in OC cell viability[22]. In our study, *AKT1* was identified as one of the hub genes with the most connections in high-grade OC (Stage IV). This finding correlates with the literature and emphasizes that the overexpression may play a role in mediating the progression and metastasis of ovarian tumors.

According to the KEGG pathway analysis, when we look at the top 5 pathways most associated with Hub genes, we observe the PGs in cancer pathway in stages II and IV. In Stage IV, the pathways of endometrial cancer and human cytomegalovirus (HCMV) infection were found to be more significant than those in other stages. PGs are characterized by the covalent attachment of a specialized linear carbohydrate chain composed of repeating disaccharide units called glycosaminoglycans (GAGs). GAG types found in PGs include heparan sulfate and chondroitin sulfate. PGs play essential roles within cells and basal membranes as secreted components of the interstitial extracellular matrix (ECM). In particular, cell surface PGs serve as integral parts of signaling events, modulation of inflammation, and adhesion in the context of tumor formation.

Table 4. Gene ontology analysis of hub genes according to the stages

	Biological process	Cellular component	Molecular function
Stage I	Positive regulation of transcription, DNA-templated	Protein-containing complex	Enzyme binding
	Positive regulation of sequence-specific DNA binding transcription factor activity	Transcription regulator complex	Disordered domain-specific binding
	Negative regulation of the apoptotic process	Nucleoplasm	Transcriptional coregulator binding
	Response to a xenobiotic stimulus	Chromatin	İdentical protein binding
	Positive regulation of the apoptotic process	Euchromatin	Protease binding
Stage II	Positive regulation of transcription, DNA-templated	Protein-containing complex	Enzyme binding
	Negative regulation of the apoptotic process	Transcription regulator complex	Disordered domain-specific binding
	Positive regulation of miRNA transcription	Chromatin	Transcriptional coregulator binding
	Positive regulation of fibroblast proliferation	Euchromatin	Identical protein binding
	Positive regulation of transcription by the RNA polymerase II promoter	Nucleus	Chromatin binding
Stage III	Positive regulation of transcription, DNA-templated	Protein-containing complex	Transcriptional coregulator binding
	Positive regulation of sequence-specific DNA binding transcription factor activity	Transcription regulator complex	Enzyme binding
	Negative regulation of the apoptotic process	Nucleus	Identical protein binding
	Response to a xenobiotic stimulus	Nucleoplasm	RNA polymerase II-specific DNA-binding transcription factor binding
	Positive regulation of the apoptotic process	Chromatin	Chromatin binding
Stage IV	Positive regulation of transcription, DNA-templated	Protein-containing complex	Enzyme binding
	Negative regulation of the apoptotic process	Transcription regulator complex	Identical protein binding
	Positive regulation of sequence-specific DNA binding transcription factor activity	Cytoplasm	Transcriptional coregulator binding
	Positive regulation of transcription by the RNA polymerase II promoter	Nucleus	Disordered domain-specific binding
	Positive regulation of gene expression	Nucleoplasm	Nitric oxide synthase regulator activity

They regulate cell-cell and cell-ECM interactions, affecting processes such as differentiation, proliferation, adhesion, and migration. Alterations in PG expression within tumor cells and the tumor microenvironment are associated with cancer progression[23]. Compared with ovarian tumors, a wider variety of heparan sulfate PGs has been found in normal ovaries. In addition, a specific type of heparan sulfate PG, syndecan-1, has been proposed to contribute to stromal induction in the pathogenesis of ovarian malignancies[24]. Understanding these changes could lead to the development of diagnostic biomarkers and more targeted therapies.

Endometrial cancer is a commonly occurring type of female reproductive system cancer originating from the lining of the uterus and is often diagnosed post-menopause. Cancer is classified into two distinct types based on biological characteristics and clinical behavior. Type I carcinoma is associated with heightened estrogen levels and is often linked to endometrial hyperplasia. It frequently displays estrogen and progesterone receptors and occurs in younger age groups. On the other hand, type II carcinoma is not linked to estrogen, often arises in the atrophic endometrium, lacks estrogen and progesterone receptors, and typically affects older individuals. Morphological disparities between these cancer types are mirrored in their molecular genetic profiles. Type I is marked by DNA mismatch repair defects and mutations in the ***PTEN***, ***K-ras***, and ***beta-catenin*** genes. In contrast, type II anemia, ***TP53*** gene mutations, and her2/neu amplification[25]. Factors such as similar histological subtypes and gene expression profiles between endometrial and ovarian cancers indicate commonalities between these two types of cancer[26]. Moreover, it has been reported that the two cancers can occur concurrently as independent tumors or metastatic tumors[27,28]. The ***PTEN***, ***beta-catenin*** (***CTNNB1***), and ***TP53*** genes highlighted in endometrial cancers were also identified as hub genes in all stages of our study. Our findings reinforce the similarities between the two cancers, and the closer relationship in Stage IV cancer suggests a potential consideration of synchronous endometrial metastasis risk.

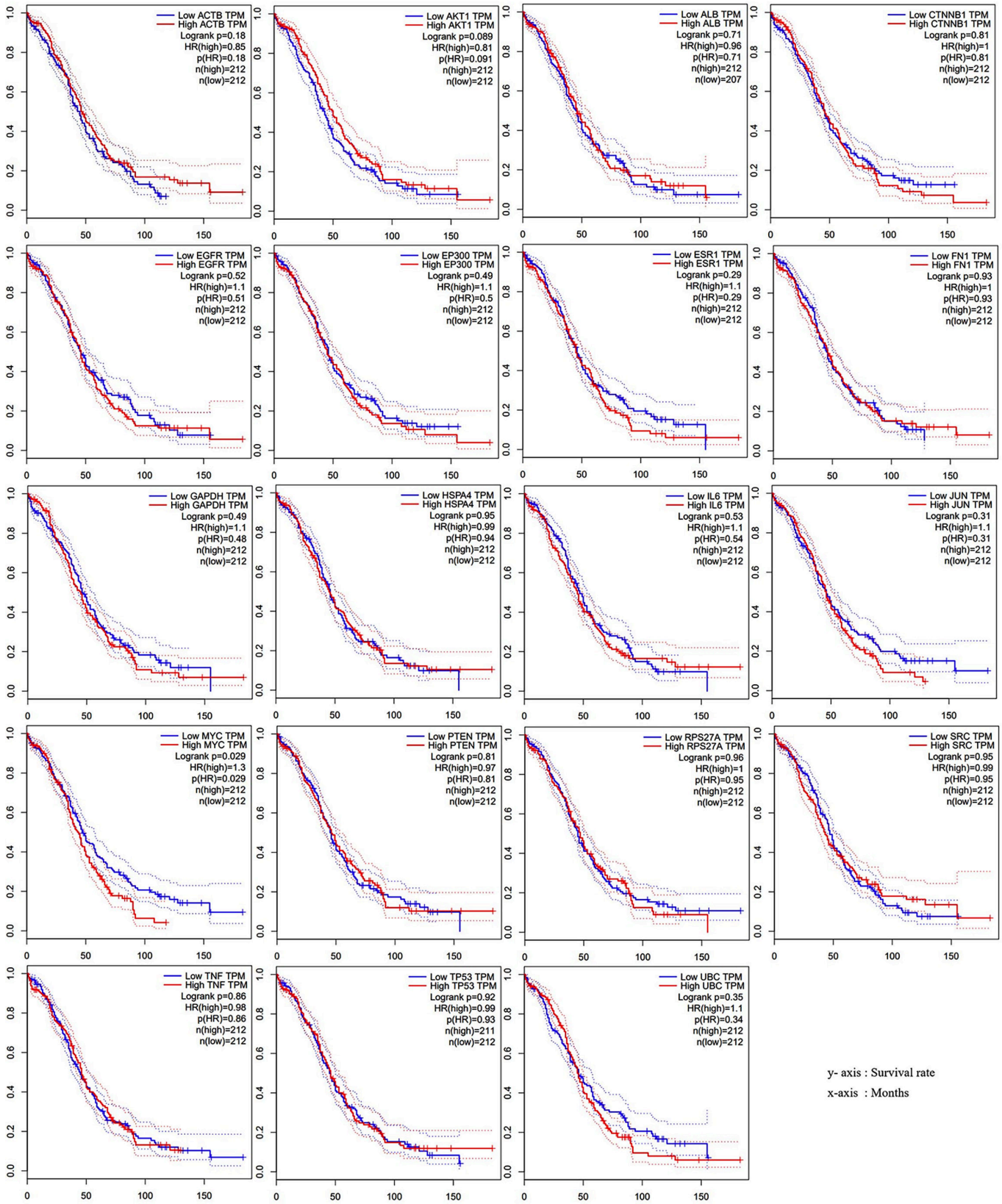

Figure 1. Survival graphs of hub genes in ovarian cancer

HR: Hazard ratio

HCMV produces approximately 200 proteins, 50 of which are crucial for replication. New ribosome profiling data suggest that over 750 unique RNA code for viral proteins. Many of these factors affect cellular and immunological functions relevant to tumor development. Recent studies indicate that the oncomodulatory properties of HCMV are important in carcinogenesis; its proteins interact with key cellular factors and pathways[29]. HCMV blocks apoptosis and evades immune surveillance, giving infected cells a survival advantage[30]. It also alters the expression of matrix metalloproteinases associated with aggressive tumors[31]. Shanmughapriya et al.[32] detected HCMV-glycoprotein B DNA in approximately 50% of OC tissues using the polymerase chain reaction. This suggests that HCMV infection in the tumor microenvironment could support cancer progression or metastasis. Intense HCMV expression is linked to shorter survival in patients with ovarian cancer, whereas higher HCMV IgG levels are associated with better prognosis[33]. The increased association of HCMV with stage IV cancer supports its link with poor prognosis. A better understanding of the oncomodulatory and immunomodulatory roles of HCMV in OC is needed. Therefore, immunotherapies could be potential targets for advanced treatment strategies in ovarian cancer.

Study Limitations

This study has limitations because of the limited sample size derived from microarray datasets and the absence of survival analysis on sufficient clinical samples. In future prospective studies with larger sample sizes, assessing the clinical significance of hub genes identified as biomarkers for ovarian cancers is crucial.

Conclusion

In conclusion, this study identified DEGs between ovarian cancers and normal ovarian tissues by analyzing seven gene expression microarray datasets. *ACTB*, *AKT1*, *ALB*, *CTNNB1*, *EGFR*, *EP300*, *ESR1*, *FN1*, *GAPDH*, *HSPA4*, *IL6*, *JUN*, *MYC*, *PTEN*, *RPS27A*, *SRC*, *TNF*, *TP53,* and *UBC* were identified as hub genes in our study. Among these hub genes, the *TP53* gene was found to have the most interactions in all stages, suggesting that *TP53* may contribute to OC development. *EGFR* was found to have the highest interactions in stage II. We suggest that EGFR is a potential biomarker for the prognosis of ovarian cancer. In our study, *RPS27A* was found to be a hub gene in stage III, suggesting the importance of *RPS27A* in tumorigenesis and OC progression. *AKT1* was identified as a hub gene with the highest number of interactions in high-grade OC (Stage IV). This finding emphasizes that the overexpression of *AKT1* may mediate the progression and metastasis of ovarian tumors. The findings of this study are expected to shed light on the development, progression, and differentiation of ovarian cancers and contribute to the development of novel therapeutic approaches through new clinical, epidemiological, and experimental studies.

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Ethics

Ethics Committee Approval: Our research is based on open-source data and therefore does not require ethics committee approval.
Informed Consent: Informed consent was obtained from all participants.
Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: B.G., D.S.A., Design: B.G., D.S.A., Data Collection or Processing: B.G., N.K.S., Analysis or Interpretation: B.G., N.K.S., D.S.A., Literature Search: B.G., N.K.S., D.S.A., Writing: B.G., N.K.S., D.S.A.
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Pre-treatment inflammatory and immune system parameters predicting cervical cancer metastasis

Rahim ağzı kanseri metastazını öngören tedavi öncesi enflamasyon ve bağışıklık sistemi parametreleri

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Abstract

Objective: This study aimed to evaluate the relationship between mannose-binding lectin-associated serine protease-2 as an immune system parameter and neutrophil lymphocyte ratio (NLR) as an inflammatory parameter to predict cervical cancer metastasis.

Materials and Methods: This cross-sectional study included 70 patients diagnosed with cervical cancer between January 2022 and February 2023 at Dr. Wahidin Sudirohusodo Hospital, Hasanuddin University Hospital, and Ibnu Sina Hospital, Makassar, Indonesia. Blood samples taken before therapy as well as clinical and histological data were gathered and examined. MASP-2 levels and NLR were measured by ELISA and flow cytometry respectively.

Results: The median age of the patients was 46 years (range, 24-72 years), with the majority of patients aged between 41 and 52 years. Statistical analysis showed that MASP-2 was associated with cervical cancer stage (p≤0.000), organ metastasis (p=0.011), and lymphovascular invasion (p=0.036). In addition, NLR was associated with cervical cancer stage (p=0.004), histopathology type (p=0.031), tumor size (p=0.019), and organ metastasis (p=0.013).

Conclusion: Pretreatment with MASP-2 as an immune system parameter and NLR as an inflammatory parameter is associated with cervical cancer metastasis. The NLR indicator can be applied in clinical practice because it is simple and reasonably priced.

Keywords: Cervical cancer, Mannose-binding lectin-associated serine protease-2, neutrophil lymphocyte ratio

Öz

Amaç: Bu çalışmada serviks kanseri metastazını öngörmek amacıyla bir bağışıklık sistemi parametresi olarak mannoz bağlayıcı lektin ilişkili serin proteaz-2 ile enflamatuvar bir parametre olarak nötrofil lenfosit oranı (NLR) arasındaki ilişkinin değerlendirilmesi amaçlandı.

Gereç ve Yöntemler: Bu kesitsel çalışmaya Ocak 2022 ile Şubat 2023 tarihleri arasında Endonezya'nın Makassar şehrindeki Dr. Wahidin Sudirohusodo Hastanesi'nde, Hasanuddin Üniversite Hastanesi'nde ve İbnu Sina Hastanesi'nde rahim ağzı kanseri teşhisi konulan 80 hasta dahil edildi. Tedaviden önce alınan kan örneklerinin yanı sıra klinik ve histolojik veriler toplanıp incelendi. MASP-2 seviyeleri ve NLR sırasıyla ELISA ve akış sitometrisi ile ölçüldü.

Bulgular: Hastaların ortanca yaşı 46 (aralık, 24-72) olup, hastaların çoğunluğunun yaşı 41 ile 52 arasındaydı. İstatistiksel analiz MASP-2'nin rahim ağzı kanseri evresi (p≤0,000), organ metastazı (p=0,011) ve lenfovasküler invazyon (p=0,036) ile ilişkili olduğunu gösterdi. Ayrıca NLR'nin rahim ağzı kanseri evresi (p=0,004), histopatoloji tipi (p=0,031), tümör boyutu (p=0,019) ve organ metastazı (p=0,013) ile ilişkili olduğu görüldü.

Sonuç: Tedavi öncesi ölçülen bir bağışıklık sistemi parametresi olarak MASP-2 ve enflamatuvar bir parametre olarak NLR serviks kanseri metastazı ile ilişkilidir. NLR basit ve uygun fiyatlı olması nedeniyle klinik pratikte bir belirteç olarak kullanılabilir.

Anahtar Kelimeler: Rahim ağzı kanseri, Mannoz bağlayıcı lektin ilişkili serin proteaz-2, nötrofil lenfosit oranı

PRECIS: Pre-treatment MASP-2 as immune systems parameters and NLR as inflammatory parameters is a simple and inexpensive indicator to predict cervical cancer metastasis.

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Introduction

Particularly in developing nations, cervical cancer continues to be a major public health issue impacting women between the ages of 40 and 55. Oncogenic human papilloma virus (HPV) infection is the main cause of cervical cancer. It can be influenced by several factors such as smoking, number of parities, changing sexual partners, marriage, or intercourse from a young age. Chronic HPV infection causes suppression of the immune response, which can disrupt the immune system[1-4].

The systemic immune inflammation index has been recognized as a reliable indicator of both systemic and local immunological responses based on peripheral neutrophil, platelet, and lymphocyte counts. The tumor microenvironment (TME) depends on several inflammatory cells and mediators. Acute-phase proteins, platelets, neutrophils, lymphocytes, and peripheral leukocytes can be observed and are involved in the inflammatory response[5].

Cell production and exudates from capillary leaks in the TME activate the complement system. It has been proposed that complement is crucial for cancer immunity. Mannose-binding lectin (MBL), a component of the complement system's lectin pathway that participates in cytolysis, opsonization, and inflammatory reactions, is a component of the natural immune system[6].

MBL interacts with antigen-presenting cells in the tumor microenvironment, which affects their activity/proliferation and thus influences the outcome of the anti-tumor immune response system. It also directly interacts with neoplasm cells by inhibiting metalloproteinases from degrading the extracellular matrix of carcinogenic agents. Interactions with MBL-associated serine protease (MASP) enable MBL to initiate the complement cascade. MASP-2, a mannose-binding lectin-associated serine proteinase, is involved in the survival and recurrence of many malignancies. A lack of MASPs is linked to an increased risk of infection, whereas an abundance of MASPs is associated to tissue damage[7,8].

The complement system will also have an impact on the TME, which will increase tumor-associated macrophages, tumor-associated neutrophils, and myeloid-derived suppressor cells, leading to the release of pro-inflammatory cytokines that increase neutrophils and suppress T-cells, NK cells, and lymphocytes. This increases neutrophil lymphocyte ratio (NLR) in the blood. Increased NLR is a marker that predisposes tumors to proliferation and metastasis through apoptosis inhibition, angiogenesis promotion, and DNA damage[9,10]. This study aims to determine how to predict cervical cancer metastasis by comparing the mannose-binding lectin-associated serine protease-2 and NLR.

Materials and Methods

Study Design

This article was generated in accordance with the strengthening the reporting of observational studies in epidemiology 2007 (STROBE)[11]. A cross-sectional study was conducted at the Hasanuddin University Hospital, Ibnu Sina Hospital, and Dr Wahidin Sudirohusodo Hospital, Makassar, Indonesia between January 2022 and February 2023.

Population and Study Setting

The participants were women newly diagnosed with cervical cancer based on histopathological examination from cervical biopsy and agreed to participate, attending the gynecology clinic during the data collection period. The following conditions had to be met for inclusion in the study: (1) Cervical cancer as determined by histopathological examination; (2) no concurrent malignancy; (3) no prior history of blood transfusion within 60 days; (4) no prior history of chemotherapy or radiation; and (5) full access to medical records. The following were the exclusion criteria: (1) being pregnant; (2) taking immunosuppressants; and (3) having a hematologic condition, an autoimmune condition, organ dysfunction, an acute or chronic infection, or another disease.

Variables

The MASP-2 level and NLR were independent variables in this study. In addition, the International Federation of Gynecology and Obstetrics (FIGO) stage[12], histopathology subtype, histological grade, tumor size, lymph node metastasis, organ metastasis, and lymphovascular invasion were the dependent variables.

MASP-2 is a zymogen that initiates the innate immune response by binding to the MBL and activating the lectin complement pathway. MASP-2 was measured using a Microplate Reader Biorad model 680 (ELISA) and expressed in ng/ml with the following cut-off values >291.9 ng/mL from the previous study[7]. Using flow cytometry analysis, NLR compares the number of neutrophils and lymphocytes from differential count calculations. The results of the comparison of the two are expressed as percentages.

The stage of cervical cancer is assessed according to the FIGO classification (2021) based on data from clinical examinations, ultrasonography, chest radiography, and computed tomography (CT) scans. Cervical cancer stages are divided into early (IA1-IB2), locally advanced (IB3-IIA2) and advanced (IIB-IVB) stages[12]. Histopathological subtypes are cell and tissue morphology types that are visible microscopically and are grouped into squamous cell carcinoma, adenocarcinoma, and adenosquamous carcinoma. Histopathological grades were grouped based on the level of cell differentiation into three categories: G1 (good differentiation), G2 (moderate differentiation), and G3 (poor differentiation). Lymphovascular invasion was assessed on the basis of the presence of intralymphatic cancer cells or intravascular cervical tissue on histopathological examination. All histopathological examinations were performed by one pathologist blinded to the patient's clinical information.

Tumor size was evaluated by rectovaginal examination, abdominopelvic ultrasound, CT, and magnetic resonance imaging (MRI) based on the largest distance in centimeters. Tumor sizes were categorized into <2 cm, 2-4 cm, >4 cm, and null (not included in the examination results). The spread of other organs is assessed on the basis of mucosal involvement of the bladder, rectum, lungs, liver, bones, and/or other organs on ultrasound examination, CT scan, and MRI.

MASP-2 Level and NLR Measurement

The two main parameters were measured using a venous blood sample (5 cc) from the cubital vein. After the blood specimen is put into the SST tube, it is inverted 5-10 times until homogeneous. The sample was sent to the Prodia laboratory for testing with the Human MASP-2 SimpleStep ELISA Kit (Abcam, Cambridge, UK). Measurements were performed using the Microplate Reader Biorad model 680 (Bio-rad Laboratories Inc., CA, USA) with Microplate Manager software ver 5.2.1 (Bio-rad Laboratories Inc., CA, USA).

Some blood samples were also taken for the NLR examination. A complete blood count was performed using an automated hematological examination device (Yumizen H1500/2500, Kyoto, Japan) to obtain a differential leukocyte count. Next, the neutrophils and leukocytes were divided to measure the NLR.

Data Collection

The patient's medical records and pathology reports were used to gather data. Clinical information for the patient included clinicopathological statistics (age, histologic grade, tumor size, lymph node metastases, lymphovascular space invasion, and FIGO stages) and the outcomes of a pre-treatment routine blood test. All patients had venous blood drawn, which was then sent to Prodia Laboratory for testing with the Human MASP-2 SimpleStep ELISA Kit Reagent and NLR, which is the neutrophil count divided by the lymphocyte count.

Sampling Size

A purposive sampling method was employed to obtain the samples. The calculation formula for single-population proportion studies was used to estimate the sample size. The sample size (n) was 62 when using a proportion of 50%, a 95% confidence interval, and a degree of precision of 10%; after adding an additional 10% for the non-response rate, the total sample size was 67.

Statistical Analysis

SPSS 24.0 was used for statistical analysis after gathering and logging the main data. The data distribution was determined using the Kolmogorov-Smirnov method, and the median and interquartile range were used to express the central tendency. For variables with two unpaired groups, the Mann-Whitney U test was employed, whereas the Kruskal-Wallis test was used for variables with more than two unpaired groups. A p-value <0.05 is the limit for determining significant test results.

Results

Subject Characteristics

There were a total of 85 patients with recently diagnosed cervical cancer. The final analysis comprised 70 patients who met the eligibility requirements (Figure 1). Table 1 displays the clinicopathological characteristics of the patients. Most patients were between the ages of 41 and 52 years, with a median age of 46 (ranging from 24 to 72). To be more precise, there were 37 cases (52.8%) who were over 45 years old and 33 cases (47.2%) who were under 45 years old.

Association Between MASP-2 and Clinicopathological Parameters

The association of MASP-2 with clinicopathological parameters is shown in Table 2. Histopathological type (p=0.798), histological grade (p=0.194), tumor size (p=0.121), and lymph node metastasis (p=0.100) were not significantly associated with MASP-2 levels. In contrast, cervical cancer stage (p≤0.000), organ metastasis (p=0.011), and lymphovascular invasion (p=0.036) were significantly associated with MASP-2 levels.

Association Between NLR and Clinicopathological Parameters

The results of the analysis of the relationship between NLR and cervical cancer clinicopathology are presented in Table 3. Statistical results showed no significant correlation between NLR level and histological grade (p=0.718), lymph node metastasis (p=0.404), and lymphovascular invasion (p=0.992), whereas cervical cancer stage (p=0.004), histopathology type (p=0.031), tumor size (p=0.019), and organ metastasis (p=0.13) showed significant association with NLR.

Discussion

This study shows that MASP-2 levels were associated with cervical cancer stages statistically. In the pathophysiology of cancer, the complement system can have two functions. Initially, it can regulate the removal of cells that can potentially cause

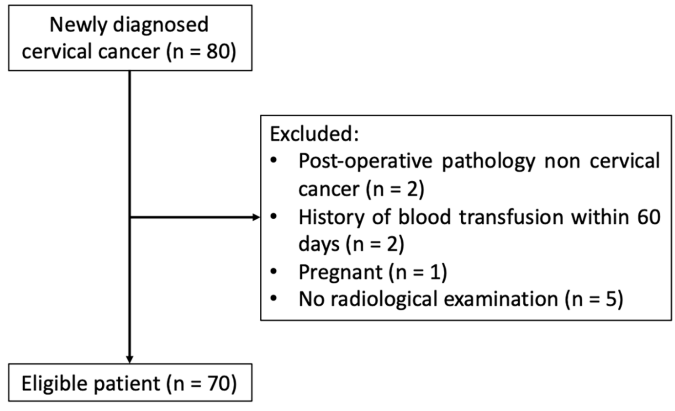

Figure 1. Flowchart of patient screening

Table 1. The clinicopathological characteristics of patient

Characteristics	n*	(%)
FIGO stage		
Stage I		
IA1	0	0
IA2	0	0
IB1	2	3.0
IB2	1	1.0
IB3	2	3.0
Stage II		
IIA1	1	1.0
IIA2	0	0
IIB	31	44.0
Stage III		
IIIA	2	3.0
IIIB	22	31.0
IIIC1	4	6.0
IIIC2	0	0
Stage IV		
IVA	4	6.0
IVB	1	1.0
Histopathology		
Squamous cell carcinoma	60	85.7
Adenocarcinoma	7	10.0
Adenosquamous carcinoma	3	4.3
Histological grade		
G1	15	21.4
G2	30	42.9
G3	25	35.7
Tumor size		
<2 cm	-	-
2-4 cm	11	15.7
>4 cm	47	67.1
Unknown	12	17.1
Lymph node metastasis		
Positive	24	34.3
Negative	46	65.7
Organ metastasis		
Positive	28	40.0
Negative	42	60.0
Lymphovascular invasion		
Positive	13	18.6
Negative	31	44.3
Unknown	26	37.1

*n=70, FIGO: International Federation of Gynecology and Obstetrics

tumors and have dangers or pathogen-associated molecular patterns or pathogens (DAMPs or PAMPs). Furthermore, complement activation may promote the growth of tumors[6]. Mannan-binding lectin (MBL) and collectin-LK, as well as ficolins, are pattern recognition molecules that can bind to carbohydrates on the surface of microbes or damaged tissue and activate the complement system through the lectin pathway. When the aforementioned occurs, two MBL-associated proteins (MAp44 and MAp19) serve as endogenous competitive inhibitors, whereas three MBL-associated proteases (MASP-1, -2, and -3) activate the complement system. Tumor tissue may release mediators that can increase serum MASP-2 levels, which are associated with poor prognosis, disease recurrence, and mortality in cervical cancer[7,8]. MASP-2 levels may facilitate or inhibit malignancy progression through complement factor C5a, which may influence the microtumor environment. However, the observed MASP-2 levels may also result from chronic inflammation, which is an ongoing acute phase associated with tumor growth[13-15].

This study also found that NLR was associated with cervical cancer stages. Neutropenia, thrombocytosis, and lymphocytopenia are signs that cancer has progressed to an advanced stage. As a result, the interaction of lymphocytes, neutrophils, and platelets has been investigated as a prognostic factor in predicting the course of malignancy. Previous studies have illustrated that an increase in NLR aligns with more advanced severity (advanced stage)[9,10].

This study showed no statistical association between MASP-2 levels and the histopathological type of cervical cancer. There was an association between NLR and the histological type of cervical cancer. However, MASP-2 levels were highest in adenosquamous carcinomas, followed by adenocarcinoma types and squamous cell carcinomas.

Histologically, squamous cell carcinoma is the disease's most prevalent subtype, followed by adenocarcinoma of the cervix, with a percentage of approximately 75% and 15%, respectively. Adenosquamous carcinoma and neuroendocrine tumors are uncommon histological subtypes. Cervical adenosquamous carcinoma is a rare malignant epithelial neoplasm characterized by squamous and glandular cell differentiation[16]. In more than 80% of instances, HPV infection -particularly HPV type 16 and HPV 18- causes cervical cancer. The virus can stimulate the complement system cascade, which is fully activated only during the inflammatory process, opsonization, phagocytosis, and pathogen elimination. This process activates the adaptive immune response and promotes cancer development[17]. In contrast to HPV-positive cervical cancer, people with HPV-negative cervical carcinoma are discovered more frequently at an advanced stage and frequently have non-squamous histology. The difference in prognosis between HPV-positive and HPV-negative cervical cancer depends on histology and not HPV status[18,19]. According to the limited number of

Table 2. Association of MASP-2 with clinicopathological parameters

			MASP-2	
		n	Median (IQR)	p-value
FIGO stage	Early	3	256.88 (244.69-323.71)	<0.000**
	Locally advanced	3	274.69 (257.34-286.42)	
	Advance	64	639.68 (485.47-738.24)	
Histopathology	Squamous cell carcinoma	60	590.09 (447.11-740.3)	0.798**
	Adenocarcinoma	7	697.26 (573.61-709.91)	
	Adenosquamous carcinoma	3	732.50 (540.93-735.78)	
Histological grade	G1	15	586.35 (465.22-696.87)	0.194**
	G2	30	549.25 (443.22-737.42)	
	G3	25	703.56 (550.46-754.82)	
Tumor size	<2 cm	-	-	0.121**
	2-4 cm	10	549.25 (461.85-653.88)	
	>4 cm	48	579.57 (424.19-741.12)	
	Unknown	12	704.74 (654.18-762.78)	
Lymph node metastasis	Positive	24	700.02 (507.35-749.00)	0.100*
	Negative	46	566.83 (414.28-723.56)	
Organ metastasis	Positive	28	704.74 (520.65-798.71)	0.011*
	Negative	42	555.66 (430.79-698.83)	
Lymphovascular invasion	Positive	13	703.56 (653.88-791.35)	0.036**
	Negative	31	560.87 (405.48-695.76)	
	Unknown	26	600.69 (465.22-743.18)	

*Mann-Whitney U, **Kruskal-Wallis, IQR: Interquartile range, FIGO: International Federation of Gynecology and Obstetrics, MASP: Mannose-binding lectin-associated serine protease

studies investigating the association between MASP-2 and histopathological types of cervical cancer, the present results can be used for further research to determine the cut-off for MASP-2.

Previous studies have shown that adenosquamous cervical carcinoma has the worst outcomes[20,21]. In addition, most studies examining NLR as a predictor of cervical cancer patient survival reported no association between NLR and histological type[22-24]. The present study had contrary findings and may be argued as follows: (1) The number of cases included was different, the size was small, (2) There were significant differences in baseline characteristics between the two groups, and (3) The comparison of the number of the three histopathological types was significantly different.

This study showed no statistical relationship between MASP-2 and NLR levels and the degree of cervical cancer differentiation but MASP-2 and NLR levels were found to be higher in poor differentiation than in good differentiation. The results demonstrate that MASP-2 and NLR can facilitate neoplastic transformation by triggering unregulated complement activation, which increases tumor cell proliferation, motility, and invasiveness as well as angiogenesis, growth factor synthesis, and host suppression[25]. Poor differentiation indicates a worse prognosis than excellent differentiation. According to this study's NLR findings and Wang et al.'s research[26], there is no association between NLR and the degree of differentiation of cervical cancer. Further study should be conducted to identify the cut-off value of MASP-2 to predict cervical cancer differentiation.

This study showed no statistical relationship between MASP-2 levels and tumor size, but MASP-2 levels were higher in tumors measuring more than 4 cm than those measuring less than 4 cm. In addition, there was an association between NLR and tumor size. A previous study revealed that patients with a tumor size 2 cm showed an almost two-fold lower risk of mortality from cervical cancer than patients with tumors measuring 2-4 cm, and tumors >4 cm showed unexpected distant metastasis[27]. This may suggest that excessive inflammation activates the complement system, resulting in increased levels of MASP-2, which activates the lectin pathway and releases proinflammatory cytokines that stimulate an increase in neutrophils and suppress lymphocytes, which increase the proliferation, migration, and

Table 3. Association of NLR with clinicopathological parameters

			NLR	
		Median (IQR)	Median (IQR)	p-value
FIGO stage	Early	3	2.03 (1.51-2.09)	0.004**
	Locally advanced	3	1.53 (1.46-2.34)	
	Advance	64	4.2 (2.97-7.71)	
Histopathology	Squamous cell carcinoma	60	4.02 (2.81-7.96)	0.031**
	Adenocarcinoma	7	2.58 (2.13-3.04)	
	Adenosquamous carcinoma	3	4.94 (4.68-5.53)	
Histological grade	G1	15	3.60 (2.52-6.42)	0.718**
	G2	30	3.86 (3.01-8.00)	
	G3	25	3.91 (2.58-5.70)	
Tumor size	<2 cm	-	-	0.019**
	2-4 cm	10	2.18 (1.49-3.77)	
	>4 cm	48	3.89 (2.88-7.71)	
	Unknown	12	4.73 (3.84-7.06)	
Lymph node metastasis	Positive	24	4.67 (2.43-11.73)	0.404*
	Negative	46	3.81 (2.83-5.62)	
Organ metastasis	Positive	28	4.52 (3.18-13.64)	0.013*
	Negative	42	3.51 (2.29-4.94)	
Lymphovascular invasion	Positive	13	3.48 (3.15-8.73)	0.992**
	Negative	31	4.42 (2.62-6.81)	
	Unknown	26	3.83 (3.02-6.96)	

*Mann-Whitney U, **Kruskal-Wallis. IQR; Interquartile range, FIGO: International Federation of Gynecology and Obstetrics, NLR: Meutrophil lymphocyte ratio

invasion activities of tumor cells[28]. This finding is also in line with that of Huang et al.[29], who explained that NLR is positively associated with tumor size. The insignificant results for MASP-2 levels in this study may be related to tumor sizes that had not been identified through radiological examination. Although there was no statistically significant correlation between MASP-2 and NLR levels and lymph node spread in this study, participants with lymph node spread had higher MASP-2 and NLR levels. This study found an association between MASP-2 and NLR levels and organ metastasis, but not between NLR and LVSI involvement. However, there was an association between MASP-2 levels and LVSI involvement. The activation of the complement system is part of the immune system, which also affects the TME and stimulates the proliferation of macrophages and neutrophils and suppresses myeloid cells, thereby stimulating the release of pro-inflammatory cytokines, which increase neutrophil proliferation and suppress lymphocytes, T-cells, and NK cells. Therefore, elevated blood NLR is a marker that predisposes tumors to proliferation and metastasis by inhibiting apoptosis, promoting angiogenesis, and causing DNA damage[17]. An earlier study also discovered a relationship between NLR and carcinoma metastasis and higher MASP-2 levels in unsurvived patients as opposed to those who survived[7]. The predictive value of NLR is highly substantial for LVSI, which contrasts with the findings of this study[30,31].

Study Limitations

The insignificant results in this study may be due to some histopathological results not including the presence or absence of LVSI involvement, thus possibly contributing to the results obtained. Recently, no study has linked MASP-2 levels with LVSI involvement; therefore, this study can be used to develop MASP-2 cut-off values.

Conclusion

This study showed that pretreatment with MASP-2 as an immune system parameter is significantly associated with organ metastasis and lymphovascular invasion in cervical cancer. Moreover, NLR as an inflammatory parameter is significantly associated with FIGO stage, histopathology, tumor size, and organ metastasis in cervical cancer. The NLR indicator can be applied in clinical practice because it is simple and reasonably priced.

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Ethics

Ethics Committee Approval: Ethical approval was obtained from Institute for Research and Community Services LPPM Hasanuddin University, protocol number UH21090607 and date of approval 16 November 2021.
Informed Consent: Informed consent was obtained.
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Authorship Contributions

Surgical and Medical Practices: M.A., N.U.P., A.R., Concept: N.U.P., Design: M.A., N.U.P., A.R., Data Collection or Processing: M.A., N.U.P., A.R., S.R., N.R., Analysis or Interpretation: M.A., N.U.P., A.R., S.R., N.R., F.H., Literature Search: M.A., Writing: M.A., N.U.P., A.R., S.R., N.R., F.H.
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Review / Derleme

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What is the effect of sildenafil citrate intake on women undergoing assisted reproduction? A systematic review and meta-analysis of randomized controlled trials

Sildenafil sitrat alımının yardımcı üreme teknikleri uygulanan kadınlar üzerinde etkisi nedir? Randomize kontrollü çalışmaların sistematik bir incelemesi ve meta-analizi

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Abstract

Assisted reproductive technologies (ART) have become a vital option for women facing fertility challenges. One of the potential interventions being explored is the use of sildenafil citrate (SC) to improve clinical outcomes in ART procedures. The aim of this study was to assess the impact of SC on clinical outcomes in women undergoing ART. A comprehensive literature search was conducted using multiple databases, including PubMed, Scopus, Embase, Web of Science, and the Cochrane Central Register of Controlled Trials. The search covered studies from inception until April 15, 2023, and identified relevant randomized controlled trials (RCTs) for inclusion in the analysis. The endpoints were summarized as risk ratio (RR) or standardized mean difference (SMD) with 95% confidence interval (CI). After meticulous analysis, twenty-eight RCTs comprising 3,426 women were included in the study. The results revealed significant findings regarding the impact of SC on clinical pregnancy (CP) rates. Women receiving SC demonstrated a significantly higher probability of CP compared to the control group (n=21 RCTs, RR=1.43; 95% CI: 1.29, 1.59). Additionally, when SC was combined with other medications like clomiphene citrate (CC) or estradiol valerate, it further improved the likelihood of CP compared to these medications alone (RR=1.35, 95% CI: 1.19, 1.53; RR=1.55, 95% CI: 1.08, 2.22, respectively). Furthermore, the study observed that the mean endometrial thickness (ET) was significantly higher in women who received SC compared to the control group, which involved other active interventions or placebo (SMD=0.77, 95% CI: 0.20, 1.34). Particularly, the administration of SC resulted in a notably higher ET level compared to the placebo (SMD: 1.33, 95% CI: 0.15, 2.51). The findings suggest that luteal supplementation of SC can be considered a beneficial approach to enhance ET and improve the CP rate in women undergoing ART.

Keywords: Assisted reproduction technology, sildenafil citrate, endometrial thickness, chemical pregnancy, clinical pregnancy

Öz

Yardımcı üreme teknolojileri (YÜT), doğurganlık sorunları yaşayan kadınlar için hayati bir seçenek haline geldi. Araştırılan potansiyel müdahalelerden biri, YÜT prosedürlerinde klinik sonuçları iyileştirmek için sildenafil sitratın (SS) kullanılmasıdır. Bu çalışmanın amacı, YÜT uygulanan kadınlarda SS'nin

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klinik sonuçlar üzerindeki etkisini değerlendirmektir. PubMed, Scopus, Embase, Web of Science ve Cochrane Central Register of Controlled Trials dahil olmak üzere birçok veri tabanı kullanılarak kapsamlı bir literatür araştırması yapıldı. Araştırma, başlangıçtan 15 Nisan 2023'e kadar olan çalışmaları kapsadı ve analize dahil edilecek ilgili randomize kontrollü çalışmaları (RKÇ'ler) belirledi. Sonlanım noktaları, risk oranı (RR) veya %95 güven aralığı (GA) ile standartlaştırılmış ortalama fark (SOF) olarak özetlendi. Titiz bir analizin ardından çalışmaya 3.426 kadını kapsayan 28 RKÇ dahil edildi. Sonuçlar SS'nin klinik gebelik (KG) oranları üzerindeki etkisine ilişkin önemli bulgular ortaya çıkardı. SS alan kadınlar, kontrol grubuyla karşılaştırıldığında anlamlı olarak daha yüksek KG oranı gösterdi (n=21 RKÇ, RR=1,43; %95 GA: 1,29, 1,59). Ek olarak SS, klomifen sitrat veya östradiol valerat gibi diğer ilaçlarla birlikte kullanıldığında, KG olasılığını bu ilaçların tek olarak kullanımı ile karşılaştırıldığında daha da artırdı (sırasıyla, RR=1,35, %95 GA: 1,19, 1,53; RR=1,55, %95 GA: 1,08, 2,22). Ayrıca bu çalışmada, diğer aktif müdahaleleri veya plaseboyu içeren kontrol grubuyla karşılaştırıldığında SS alan kadınlarda ortalama endometrial kalınlığın (EK) anlamlı derecede daha yüksek olduğu gözlemlendi (SOF=0,77, %95 GA: 0,20, 1,34). Özellikle SS'nin uygulanması, plaseboya kıyasla belirgin şekilde daha yüksek bir EK seviyesiyle sonuçlandı (SOF: 1,33, %95 GA: 0,15, 2,51). Bulgular, SS'nin luteal takviyesinin, YÜT uygulanan kadınlarda EK'yi artırmak ve KG oranını iyileştirmek için yararlı bir yaklaşım olarak değerlendirilebileceğini göstermektedir.

Anahtar Kelimeler: Yardımcı üreme teknolojisi, sildenafil sitrat, endometrial kalınlık, kimyasal gebelik, klinik gebelik

Introduction

The global use of advanced techniques such as assisted reproductive technology (ART) has expanded, leading to noteworthy advancements in the treatment of infertility. However, despite these achievements, complex challenges persist in comprehending the intricate process of implantation and enhancing the outcomes of ART. Further research and advancements are necessary to address these complexities and improve the overall success of ART procedures[1].

It is crucial to conduct additional research on current therapeutic approaches to augment the success rates of ART due to the consistently low worldwide frequencies of embryo implantation and gestation[2]. Among the intricate aspects of ART, implantation is one of the most vulnerable and complex processes[3]. It depends on numerous local and systemic elements, including immune agents and hormonal cues[4]. Before being implanted, the embryo needs to produce substances that encourage the attachment site, whereas the decidua, conversely, should emit substances that support the differentiation and initial growth of the embryo. Consequently, exploring and understanding these factors are crucial for improving the success of ART[5,6]. Synchronization of endometrial receptivity and embryonic competence in a timely manner is of utmost importance for successful embryo implantation. It is crucial that the mentioned mediators operate within a normal range and at the appropriate time to ensure optimal conditions for this process[7]. Achieving adequate endometrial growth is a vital requirement for successful implantation. However, the precise understanding of the factors influencing endometrial growth remains limited and requires further investigation[8].

In recent times, significant numbers of studies have focused on investigating angiogenesis and vascularization within the endometrium. These studies have revealed that women with thin endometrium often exhibit poor uterine receptivity, which is potentially attributable to compromised blood flow impedance through the endometrium[9]. Over the past 10 years, several approaches have been examined to improve endometrial thickness among poor endometrial responders[10-14]. Along these lines, sildenafil citrate (SC) has been extensively examined in previous clinical trials to gage its impact on enhancing the success rate of ART. This is attributed to its well-documented vasodilatory and antithrombotic activities, making it one of the most thoroughly researched medications in this regard[15,16]. Numerous clinical trials have been conducted, and a mounting body of evidence suggests a beneficial effect of SC on ART outcomes[17-19].

To date, 28 randomized clinical trials (RCTs) have been performed to investigate the effect of SC on ART outcomes. However, despite these efforts, a definitive conclusion regarding its efficacy remains elusive, and prior studies have not yielded strong evidence[20]. Considering the substantial debate surrounding the effectiveness of SC, we conducted a contemporary systematic review and meta-analysis of RCTs specifically targeted at appraising the influence of SC on various clinical endpoints of ART.

Methods

This investigation was conducted following the guidelines outlined in the preferred reporting items for systematic reviews and meta-analyses statement[21] and the Cochrane handbook for systematic reviews of interventions[22]. This investigation was registered in the international prospective register of systematic reviews under the identifier CRD42023433884.

Databases and Search Strategy

To identify potential studies, a systematic search was conducted using several databases, including PubMed, Scopus, Embase, Web of Science, and the Cochrane Central Register of Controlled Trials. The search encompassed the period from the inception of each database to April 15, 2023. In addition, we manually inspected the reference lists of pertinent published studies with the intention of discovering any extra suitable RCTs. The search query comprisedthe following: ("in vitro fertilization" OR "intracytoplasmic sperm injection" OR "IVF" OR "Embryo Transfer" OR "ICSI" AND "Sildenafil" OR "SC" OR "Hydroxyhomosildenafil" OR "Revatio" OR "Homosildenafil" OR "Acetildenafil" OR "Viagra"). Supplement Table 1 provides comprehensive information regarding the search strategy employed, including the specific terms used and database-specific indexing terminology. No limitations were set based on the year of publication or language during the search process.

Table 1. Study characteristics

Study	Country	Study design	Population	Sample size		Intervention(s)	Control	Outcome measures
				Case	Control			
Moini et al.[17] 2020	Iran	Randomized clinical trial	Women had normal ovarian reserve with at least two prior consecutive failed IVF/ICSI attempts with at least a transfer of two good quality fresh or frozen-thawed embryos	22	22	Sildenafil (vaginal suppositories, 100 mg/day, were administered from the first day of the FSH injection until the day of oocyte retrieval.	Placebo (vaginal suppositories)	Chemical pregnancy, clinical pregnancy, miscarriage, endometrial thickness implantation rate,
El-Sayed et al.[25] 2017	Egypt	Randomized clinical trial	Women have experienced two or more implantation failure attributed to inadequate endometrial development. Age 20-40 years, (BMI):20-29	40	40	Oral sildenafil citrate at dose 25 mg tab/6 h daily from day six of induction of ovulation until day of HCG administration	Nothing	Endometrial thickness
Tehraninejad et al.[43] 2018	Iran	Randomized clinical trial	Women who had previously at least two IVF failure attempts and women aged below 45 years of age.	36	36	100 mg vaginal sildenafil suppositories daily, starting on day 3 of menstruation	Routine medication for frozen thawed cycle	Clinical pregnancy
Ataalla et al.[26] 2018	Egypt	Randomized clinical trial	Patients with previous low response to controlled ovarian hyper stimulation using antagonist protocol.	30	30	Sildenafil 50 mg/day orally	Placebo 50 mg/day orally	Chemical pregnancy, clinical pregnancy, endometrial thickness implantation rate,
Wafa et al.[24] 2019	Egypt	Randomized clinical trial	Women have experienced two or more implantation failure attributed to inadequate endometrial development, age 18-35 years.	35	35	Sildenafil 25 mg orally twice daily	Nothing	Clinical pregnancy
Kortam et al.[27] 2018	Egypt	Randomized clinical trial	Unexplained infertility, 18-35 years.	45	45	CC 100 mg/d from 2nd to 6th day of cycle, oral sildenafil citrate 25 mg every 8 h from 2nd day of the cycle	CC 100 mg/d from 2nd to 6th day of cycle	Chemical pregnancy, endometrial thickness
Reddy et al.[38] 2016	India	Randomized clinical trial	Age less than 40 years and more than 18 years, primary or secondary infertility, with regular menstrual cycles, and normal semen parameters of the husband	40	40	CC 100 mg/d from 3nd to 7th day of cycle, oral sildenafil citrate Sildenafil (Viagra, Pfizer) 25 mg twice daily was given from day 8 up to ovulation trigger.	CC 100 mg/d from 3nd to 7th day of cycle	Chemical pregnancy, endometrial thickness
AbdelKader Fahmy et al.[28] 2015	Egypt	Randomized clinical trial	Women aged between 18 and 40 years with primary or secondary infertility and with regular menstrual cycles.	35	35	CC 50 mg orally 3 times/day from 3rd to 7th day of the cycle with sildenafil citrate 25 mg (Viagra, Pfizer) orally 3times/day from	CC 50 mg orally 3 times/day from 3rd to 7th day of the cycle	Chemical pregnancy, endometrial thickness

Ashoush and Abdelshafy[29] 2019	Egypt	Randomized clinical trial	PCOS women, aged 21-35 years, with clomiphene failure	239	278	Clomiphene citrate 50 mg, on the 2nd day of the menstrual cycle for 5 days for a maximum of six induction cycles. 25 mg of sildenafil citrate orally every 6 hours till the end of the cycle	Clomiphene citrate 50 mg, on the 2nd day of the menstrual cycle for 5 days for a maximum of six induction cycles	Clinical pregnancy, endometrial thickness
Aboelroose et al.[30] 2020	Egypt	Randomized clinical trial	Infertile women with primary or secondary infertility aged 18-40 years	40	40	100 mg clomiphene citrate in tablet form orally once daily from days 3-7 of the cycle and 25 mg sildenafil citrate orally twice daily from days 8-12 of the same cycle.	100 mg clomiphene citrate in tablet form orally once daily from days 3-7 of the cycle	Clinical pregnancy, endometrial thickness
Yavangi et al.[44] 2019	Iran	Randomized clinical trial	Infertile women with primary or secondary infertility	35	35	25 mg vaginal sildenafil four times a day+6 mg E2 from the second or third day of the cycle	6 mg E2 from the second or third day of the cycle	Chemical pregnancy
Vardhan et al.[39] 2019	India	Randomized clinical trial	Infertile women with primary or secondary infertility	40	40	From the first day to the fourth day, 2 mg estradiol valerate tablets, and from the 5th to the 8th day, 4 mg estradiol tablets, and from the 9th to the 12th day of the menstrual cycle, 6 mg estradiol valerate and sildenafil citrate tablets orally 25 mg TDS daily from day 1 of the cycle until the 12th day.	From the first day to the fourth day, 2 mg estradiol valerate tablets, and from the 5th to the 8th day, 4 mg estradiol tablets, and from the 9th to the 12th day of the menstrual cycle, 6 mg estradiol valerate	Chemical pregnancy, clinical pregnancy, endometrial thickness
Mangal and Mehirishi[40] 2019	India	Randomized clinical trial	Infertile women with primary or secondary infertility	50	50	Sildenafil citrate 25 mg vaginally every 6 hours for 5 days from day 8th of the cycle and tablet estradiol valerate 2 mg 6-8 hourly	Tablet estradiol valerate 2 mg 6-8 hourly	Clinical pregnancy, endometrial thickness
Dehghani Firouzabadi et al.[19] 2019	Iran	Randomized clinical trial	Infertile women with primary or secondary infertility	40	40	First to the fourth day of the menstrual cycle, 2 mg estradiol valerat tablets, from the 5th to the 8th day of the menstrual cycle, 4 mg estradiol valerat tablets, and from the 9th to the 12th day of the menstrual, 6 mg estradiol valerat tablets were given daily and sildenafil citrate tablets (50 mg) daily	First to the fourth day of the menstrual cycle, 2 mg estradiol valerate tablets, from the 5th to the 8th day of the menstrual cycle, 4 mg estradiol valerat tablets, and from the 9th to the 12th day of the menstrual, 6 mg estradiol valerat tablets were given daily.	Clinical pregnancy, endometrial thickness

Alieva et al.[48] 2012	Russia	Randomized clinical trial	Women with tubal infertility who had undergone at least 2 unsuccessful IVF and embryo transfer attempts when transferred embryos were of high quality and disturbances in uterine hemodynamics were present	23	25	Sildenafil citrate in the IVF cycle	Nothing	Clinical pregnancy, endometrial thickness
Kim et al.[49] 2010	Korea	Randomized clinical trial	Women with a thin endometrium (<8 mm: range 5 to 7.9 mm) at the time of embryo transfer undergoing IVF	21	27	Vaginal sildenafil 25 mg/d + oral estradiol valerate 4 mg/d from day of embryo transfer until pregnancy test (11 days)	Nothing	Clinical pregnancy
Abdullah et al.[45] 2021	Iraq	Randomized clinical trial	Infertile female patients who undergo stimulated intra-uterine insemination	25	25	Sildenafil citrate 50 mg vaginally every 12 hours from day 5 of the cycle for 8 days + letrozole 5 mg at cycle day 2 for 5 days	Letrozole 5 mg at cycle day 2 for 5 days	Clinical pregnancy, endometrial thickness
Belapurkar et al.[42] 2022	India	Randomized clinical trial	Infertile women with primary or secondary infertility	35	35	Self-administer non-clinically vaginal sildenafil citrate 25 mg every six hours from day seven to day 12 of the menstrual cycle	intrauterine G-CSF injection (300 mcg/1 mL) four days after the last day of menses	Endometrial thickness
Mohammed et al.[46] 2023	Iraq	Randomized clinical trial	Infertile women who were undergoing intracytoplasmic sperm injection	30	30	Sildenafil citrate 25 mg vaginally every 6 hours from day of stopping of cycle to day of HCG	Nothing	Clinical pregnancy
Dawood et al.[47] 2020	Iraq	Randomized clinical trial	Women with unexplained, primary or secondary infertility who have thin endometrium	30	30	Sildenafil citrate 25 mg vaginally every 6 hours	Estradiol valerate 2 mg tablet 12 hourly	Clinical pregnancy, Endometrial thickness
Mohamed et al.[18] 2022	Eygept	Randomized clinical trial	PCOS women, aged 20-35 years, with primary or secondary infertility	50	50	Sildenafil (seldin 25 mg tab), oral tab was given every 12 hours every day starting from day 3 of the period till leading follicle size reaches about 18-20 mm + clomiphene citrate (clomid 50 mg tab) two tablets every 24 hours	Clomiphene citrate (clomid 50 mg tab) two tablets every 24 hours	Clinical pregnancy
Gupta et al.[41] 2021	India	Randomized clinical trial	Infertile women with primary or secondary infertility	38	42	Sildenafil citrate 25 mg vaginally every 6 hours + 50 mg clomiphene citrate in tablet form orally once daily from days 1-5 of the cycle	50 mg clomiphene citrate in tablet form orally once daily from days 1-5 of the cycle	Clinical pregnancy

Abbas et al.[32] 2021	Eygept	Randomized Clinical trial	Infertile women with PCOS women undergoing induction of ovulation	216	216	Sildenafil citrate 25 mg vaginally every 6 hours + 50 mg clomiphene citrate in tablet form orally once daily from days 1-5 of the cycle	50 mg clomiphene citrate in tablet form orally once daily from days 1-5 of the cycle	Clinical pregnancy
ELkhouly et al.[33] 2022	Eygept	Randomized clinical trial	Infertile women aged between 18 and 35 years old diagnosed with PCO	100	100	Patients who were given sildenafil, 20 mg coated tablets from the seventh day to 1^{1th} day of the cycle three times per day orally + clomiphene citrate 50 mg was given from the 3rd day to seventh day of the same cycle two times per day orally.	Patients who were given clomiphene citrate 50 mg from the third day to seventh day of the cycle two times per day orally	Chemical pregnancy, live birth and miscarriage
El-Asbaa et al.[34] 2021	Eygept	Randomized clinical trial	Infertile women undergoing induction by clomiphene citrate attending infertility and unit	22	22	Sildenafil citrate 25 mg was given every 8 hour from 2nd day of the cycle till the day of trigger of ovulation	Oral estradiol valerate 2 mg, one tablet every 12 hour from day 8th of the cycle till triggering of ovulation	Clinical pregnancy, endometrial thickness
Abdel Hamid et al.[35] 2021	Eygept	Randomized clinical trial	Infertile women aged between 18 and 35 years with primary or secondary infertility	29	29	Vaginal sildenafil tablets 25 mg/12 h daily was given from day 8 up to ovulation trigger + 50 mg CC (clomid) orally 2 times/day from day 3 to day 7 of the cycle.	50 mg CC (clomid) orally 2 times/day from day 3 to day 7 of the cycle.	Chemical pregnancy, endometrial thickness
Wafa et al.[24] 2022	Egypt	Randomized clinical trial	Infertile women aged between 18 and 35 years with primary or secondary infertility	75	75	Sildenafil citrate, 20 mg from cycle day 8 till cycle day 13 in dose of 20 mg every 8 hours	Placebo tablets from cycle day 8 till cycle day 13 orally every 8 hours	Chemical pregnancy, endometrial thickness
El-ghany et al.[37] 2022	Egypt	Randomized clinical trial	Infertile women aged between 20 and 35 years with primary or secondary infertility	100	100	Aromatase inhibitor (letrozole 2.5 mg) 1 tablet twice daily for induction of ovulation for five-days, beginning from the 2nd day of cycle, and Sildenafil citrate: 1 tablet three times per day from with the start of Letrzole therapy until the day of HCG administration.	Aromatase inhibitor (letrozole 2.5 mg) 1 tablet twice daily for induction of ovulation for five-days, starting from the 2nd day of cycle and Placebo tablets: 1 tablet three times per day from with the start of letrzole therapy until the day of HCG administration.	Clinical pregnancy, Endometrial thickness

CC: Clomiphene citrate, PCOS: Polycystic ovary syndrome, HCG: Human chorionic gonadotropin, FSH: Follicle stimulating hormone, BMI: Body mass index, IVF: In vitro fertilization

Inclusion and Exclusion Criteria

In our review, studies were analyzed if they met the following conditions: (i) the study comprised subfertile patients undergoing ART, (ii) the intervention group received SC alone or in combination with other agents, (iii) the comparator (control) group received a placebo, no treatment or an active agent other than SC, (iv) the study reported at least one of the desired endpoints such as endometrial thickness, chemical pregnancy, clinical pregnancy, live birth or miscarriage and (v) the study design was an RCT. In contrast, our review excluded studies that were in any of the following categories: (i) the study was not an RCT, (ii) the study involved animals, and (iii) the study lacked adequate information on the study methodology or findings.

Study Selection, Data Collection, and Quality Assessment

Following the retrieval of all citations, duplicates were eliminated. Subsequently, the titles/abstracts of the residual citations were examined, and any unrelated citations were excluded. Then, the full-text citations of the residual ones were screened to establish their final eligibility. Two coauthors independently selected the studies, and any disagreements were resolved via consensus.

Data collection involved two distinct categories. The initial category encompassed the fundamental attributes of the eligible RCTs, such as the name of the primary author, year of publication, country, study arms, participant count, specifics of treatment arms, and reported results. The subsequent category comprised the clinical endpoints, namely, endometrial thickness, rate of chemical pregnancy, and rate of clinical pregnancy. The task of collecting data was carried out by two sets of coauthors who worked independently, and disputes were resolved via consensus within each pair.

The Cochrane risk of bias tool was used to assess the methodological quality of RCTs[23]. Each of the seven domains was assessed as unclear, low, or high risk of bias. Two coauthors completed the study appraisal independently, and disputes were resolved by discussion with a third co-author.

Statistical Analysis

The Mantel-Haenszel method was used to compute the risk ratio (RR) and its 95% confidence interval (CI) for the contrasting findings of chemical and clinical pregnancy rates.

In contrast, the standardized mean difference (SMD) was used to compute the continuous result of the average thickness of the endometrium, along with its 95% CI, employing the inverse-variance method. The random-effects model was employed for all calculations. The heterogeneity of the RCTs was assessed visually through forest plots and statistically using the chi-square-based Q statistic and I^2 value. Significant heterogeneity was established when the p-value of the chi-square-based Q statistic measured <0.10 or I^2 measured >50%. Stata software (version 17) was used to perform all statistical analyses. Subgroup analyses were conducted to explore the impact of SC on ART outcomes, considering related study features. These features included intervention type (e.g., SC alone, SC with clomiphene citrate (CC), SC with estradiol valerate, and SC with letrozole), population type (e.g., recurrent implantation failure (RIF) or other infertility categories), and control type (e.g., CC, estradiol, letrozole, or placebo), which were considered potential sources of variation.

Results

Summary of the Database Screening

In total, 935 records were found across all databases after eliminating duplicates. Of these, 89 records underwent a complete text examination, and among them, 61 records were excluded for specific reasons. Eventually, 28 RCTs were included in the meta-analysis (Figure 1).

Summary of Baseline Characteristics of Eligible RCTs

All RCTs had a parallel design and were performed between 2010 and 2023. The RCTs were conducted in various countries, including Egypt (n=14)[24-37], India (n=5)[38-42], Iran (n=4)[17,19,43,44], Iraq (n=3)[45-47], Russia (n=1)[48], and Korea (n=1)[49]. Seven RCTs compared SC with placebo, ten RCTs compared the combination of SC and CC with CC, 7 RCTs compared the blend of SC and estradiol valerate with estradiol valerate, three RCTs compared the combination of SC and letrozole with letrozole, and one RCT compared SC with placebo. Table 1 displays the range of sample sizes, with participant numbers varying from 44 to 850. Among the seven RCTs, the focus was on women with recurrent implantation failure. On the other hand, the remaining RCTs examined different forms

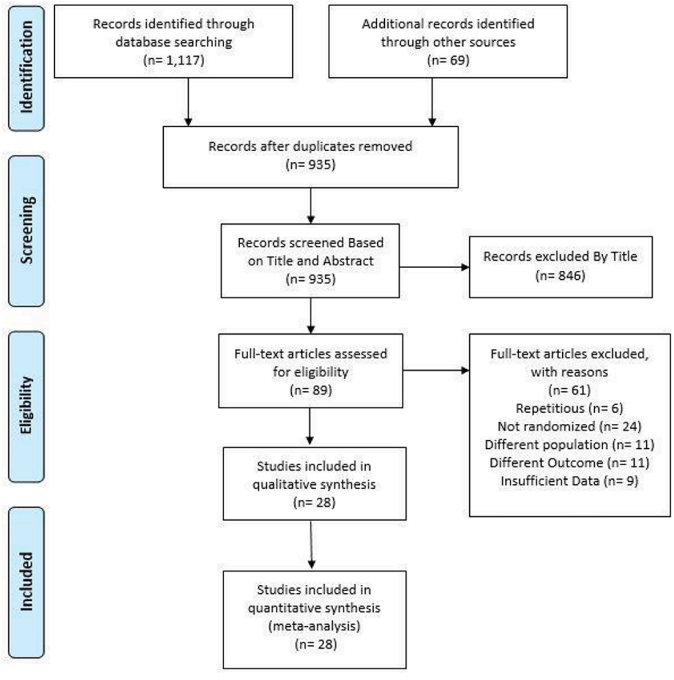

Figure 1. Flow diagram of study selection for the analysis

of infertility, including patients with narrow endometria or undetermined causes of infertility.

Summary of the Study Quality of the Included RCTs

Supplement Table 2 presents an overview of the bias risk of the RCTs that were included. With the exception of 15 RCTs, all other RCTs were determined to have a low risk regarding random generation. Uncertainty regarding random allocation was observed in 13 RCTs. For allocation concealment, 14 RCTs were deemed to have a high risk of bias, whereas eight RCTs had an unclear risk of bias. Performance bias was rated as a high and unclear risk of bias in 16 and four RCTs, respectively. Detection bias was rated as high and unclear risk of bias in 10 and 7 RCTs, respectively. Attrition bias was rated as a high and unclear risk of bias in four and six RCTs, respectively. Fifteen RCTs were concluded to exhibit an unclear risk of bias for the domain of reporting bias.

Meta-analysis of the Rate of Chemical Pregnancy

Chemical pregnancy data were pooled from 11 RCTs with a total of 982 individuals (485 cases and 497 controls). The probability of chemical pregnancy (RR=1.54; 95% CI: 1.27, 1.87) was significantly higher in the intervention group (i.e., SC alone or combination with other treatments) than in the placebo group (i.e., any other active treatment, no treatments, or placebo), without significant heterogeneity (I^2=0%; p=0.679) (Figure 2). A counter funnel plot and Egger's test showed no sign of publication bias (β=0.83, 95% CI: -1.05, 2.73; p=0.343, Supplement Figure 1). The sensitivity analysis indicated that the calculated combined risk RR ranged between 1.50 (95% CI: 1.23, 1.83) and 1.62 (95% CI: 1.32, 1.97). This suggests that none of the individual RCTs had a substantial influence on the overall effect size (Supplement Figure 2).

The impact size was more pronounced in a subgroup of RCTs that provided subcutaneous injections (SC) to RIF women (n=3 RCTs, RR=2.08, 95% CI: 1.22, 3.54, I^2=0%), compared with women with other infertility types (n=8 RCTs, RR=1.48, 95% CI: 1.20, 1.81, I^2=0%) (Supplement Figure 3). There was no distinction between the subgroup of RCTs that administered SC doses of ≤50 mg (n=5 RCTs, RR: 1.66, 95% CI: 1.25, 2.20, I^2=0%) and those that administered SC doses of more than 50 mg (n=6 RCTs, RR=1.45, 95% CI: 1.12, 1.89, I^2=0%) (Supplement Figure 4).

Three RCTs with participants experiencing RIF were conducted to compare subcutaneous SC intervention with placebo. Additionally, four RCTs compared the combination of SC and CC with CC alone, three RCTs compared the blend of SC and estradiol valerate with estradiol valerate alone, and one RCT compared the combination of SC and letrozole with letrozole alone. The forest plots, using random-effects analysis, for all the RCTs that compared SC with placebo showed a noteworthy improvement in the chemical pregnancy rate in the SC intervention group compared with the control arm (RR=2.08, 95% CI: 1.22, 3.54, I^2=0%). Furthermore, a significantly substantial change was noticed in the subgroup of RCTs that contrasted the blend of SC and CC with CC alone (RR=1.47, 95% CI: 1.15, 1.88, I^2=0%). However, no significant change was observed in the subgroup of RCTs that compared the blend of SC and estradiol valerate with estradiol valerate alone (RR=1.31, 95% CI: 0.85, 2.01, I^2=6.42%) (Figure 2).

Meta-analysis of the Rate of Clinical Pregnancy

Data on clinical pregnancy were gathered from 21 RCTs involving 2.816 patients (1.401 cases and 1.415 controls). The intervention group, which received either SC treatment alone or in combination with other therapies, exhibited a significantly higher likelihood of clinical pregnancy (RR=1.43; 95% CI: 1.29, 1.59) compared with the control group, which consisted of other active interventions, no intervention, or placebo. No significant heterogeneity was identified (I^2=2.36%; p=0.716) (Figure 3). A counter funnel plot and Egger's test indicated no evidence of publication bias (β=0.74, 95% CI: -0.01, 1.50; p=0.054, Supplement Figure 5). Sensitivity analysis demonstrated that the combined RR estimates varied from 1.40 (95% CI: 1.27, 1.55) to 1.51 (95% CI: 1.33, 1.71), indicating that no individual RCT exerted a significant influence on the overall effect size (Supplement Figure 6).

No significant changes were noticed between the subgroup of RCTs that provided SC treatment to RIF women (n=6 RCTs, RR=1.63, 95% CI: 1.13, 2.33, I^2=0%) and those with different infertility causes (n=15 RCTs, RR=1.41, 95% CI: 1.26, 1.56, I^2=72%) (Supplement Figure 7). The subset of RCTs that used SC doses of ≤50 mg (n=9 RCTs, RR=1.62, 95% CI: 1.36, 1.93, I^2=0%) showed a stronger effect size compared with those that used SC doses of more than 50 mg (n=12 RCTs, RR=1.32, 95% CI: 1.17, 1.49, I^2=0%) (Supplement Figure 8).

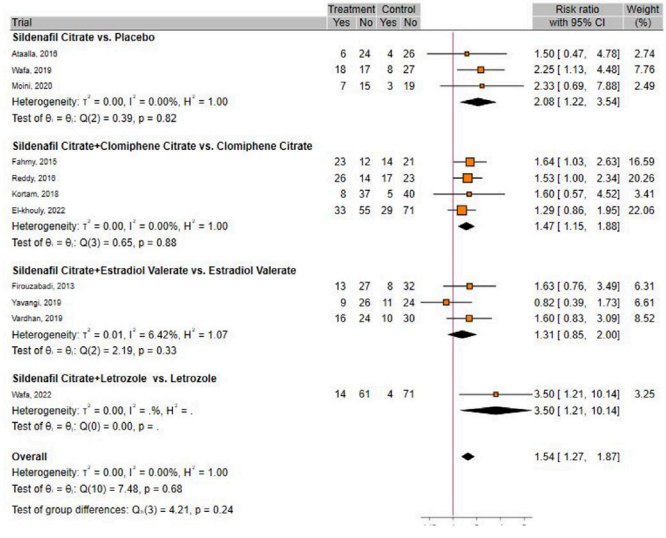

Figure 2. Forest plot showing individual and combined effect size estimates and 95% confidence intervals (CIs) in trials that evaluated the risk of chemical pregnancy in women who received sildenafil citrate versus control regarding intervention and control type

Six RCTs assessed the use of SC (n=183 patients) in contrast to a placebo (n=183 patients). Furthermore, seven RCTs investigated the combination of SC and CC (n=890 patients) compared with CC alone (n=893 patients). Six RCTs examined the combined administration of estradiol valerate and SC versus estradiol valerate alone, encompassing 203 participants in the experimental group and 209 participants in the control group. Lastly, two RCTs compared the combined use of letrozole and SC versus letrozole alone, involving 125 participants in the experimental group and 125 participants in the control group.

Pooling the outcomes of six RCTs that compared the rates of successful pregnancies between the SC (study compound) and placebo groups revealed a considerably higher likelihood of achieving clinical pregnancy in the SC group (RR=1.59, 95% CI: 1.10, 2.30, I^2=0%). Women who received both SC and CC had significantly higher chances of clinical pregnancy compared with those who received monotherapy CC (RR=1.35, 95% CI: 1.19, 1.53, I^2=0%). Remarkably, women who received both SC and estradiol valerate experienced a notable increase in clinical pregnancy rates compared with those who only received estradiol valerate (RR=1.55, 95% CI: 1.08, 2.22, I^2=0%). Conversely, no statistically significant change was observed between the intervention and control groups in a subset of RCTs that administered the combination of letrozole and SC versus letrozole alone (n=2, RR=1.78, 95% CI: 0.98, 3.24, I^2=45.42%) (Figure 3).

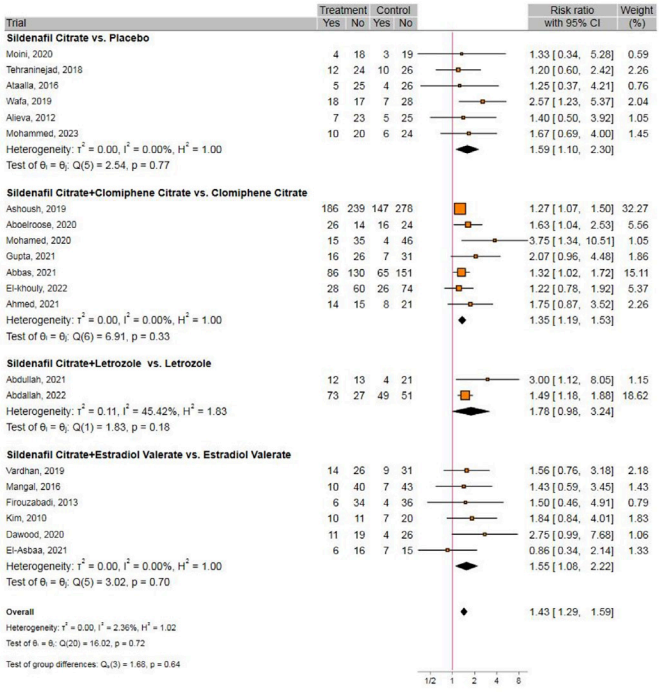

Figure 3. Forest plot showing individual and combined effect size estimates and 95% confidence intervals (CIs) in trials that evaluated the risk of clinical pregnancy in women who received sildenafil citrate versus control regarding intervention and control type

Meta-analysis of the Diameter of the Endometrial Thickness

Data regarding the thickness of the endometrium were gathered from ten RCTs involving 748 participants, with 374 being cases and 374 being controls. The findings revealed that women who received an intervention, either subcutaneous administration alone or in combination with other treatments, had a considerably higher average endometrial thickness (SMD=0.77, 95% CI: 0.20, 1.34; I^2=92.72%) than the control group. The control group included various interventions such as other active treatments, no intervention, or a placebo (Figure 4). A counter funnel plot and Egger's test were conducted, which indicated no indication of publication bias (β=5.40, 95% CI: -9.02, 19.84; p=0.413). Sensitivity analysis demonstrated that the combined RR estimates varied from 0.58 (95% CI: 0.12, 1.03) to 0.89 (95% CI: 0.39, 1.47), implying that no individual RCT had a substantial impact on the overall effect size (Supplement Figure 9).

There was a notable distinction observed between a specific group of RCTs that used SC treatment in patients experiencing RIF (consisting of 4 RCTs, with a SMD of 1.33, 95% CI: 0.15, 2.51, and an I^2 value of 94.35%) and another group that administered SC treatment to patients with different forms of infertility (comprising 6 RCTs, with an SMD of 0.43, 95% CI: -0.02, 0.88, and an I^2 value of 83.35%, Supplement Figure 10). The magnitude of the effect was more pronounced in the subset of RCTs that used SC doses exceeding 50 mg (including 7 RCTs, with an SMD of 0.65, 95% CI: 0.08, 1.22, and an I^2 value of 89.89%) than in those that employed SC doses of 50 mg (consisting of 3 RCTs, with an SMD of 1.09, 95% CI: -0.42, 2.67, and an I^2 value of 96.37%, Supplement Figure 11).

Four RCTs conducted a comparison between SC administration and placebo, with a total of 127 patients in the case group and 127 participants in the control group. Among them, one RCT compared the combined use of SC and CC (n=45 patients) with the use of CC alone (n=45 patients). Furthermore, another RCT contrasted the combined use of SC and letrozole with the use of letrozole alone, with 75 cases and 75 controls. Moreover, an additional RCT compared SC (n=35 patients) with G-CSF (n=35 patients). Lastly, one RCT compared the combined use of estradiol valerate and SC (n=40 patients) with the use of estradiol valerate alone (n=40 patients).

After the intervention, patients who were administered SC experienced an increase in endometrial thickness in comparison with those who received a placebo (n=4 RCTs, SMD=1.33, 95% CI: 0.15, 2.51, I^2=94.35%). The average endometrial thickness was significantly higher in women who received both SC and CC than in those who only received CC (n=1 RCT, SMD=0.92, 95% CI: 0.49, 1.36). No notable change between patients who received a blend of SC and estradiol valerate versus those who received estradiol valerate alone was noted (n=1 RCT, SMD=0, 95% CI: -0.43, 0.43). Additionally, women who received SC exhibited a significantly higher endometrial thickness compared

with those who received G-CSF (n=1 RCT, SMD=0.67, 95% CI: 0.19, 1.14) (Figure 4).

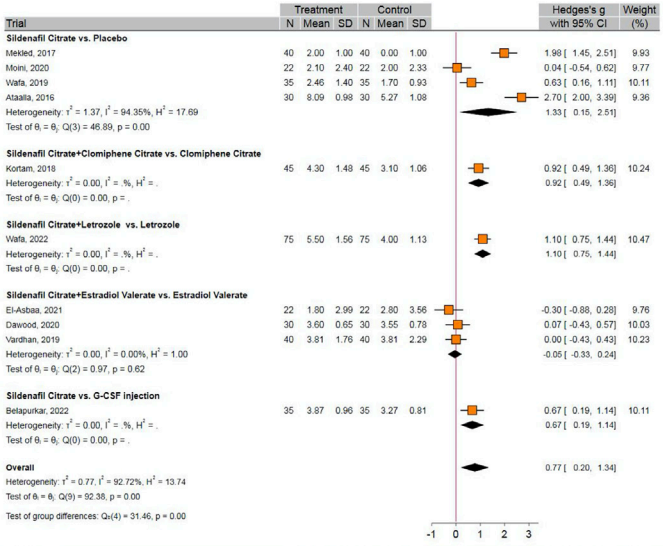

Figure 4. Forest plot showing individual and combined effect size estimates and 95% confidence intervals (CIs) in trials that evaluated the mean of endometrial thickness in women who received sildenafil citrate versus control regarding intervention and control type

SD: Standard deviation

Discussion

Understanding the specific function and operational method of SC in the implantation process is an intricate issue. However, various potential theories have been proposed. Initially, SC hinders the activity of phosphodiesterase 5 (PDE5), an enzyme responsible for breaking down cyclic guanosine monophosphate (cGMP). Through the application of SC, the levels of cGMP remain heightened, resulting in the relaxation of blood vessels and an augmented blood circulation toward the endometrium[50]. Second, SC could influence vasoactive cytokines responsible for governing the growth of the uterine lining or the attachment of the embryo[19]. Third, the use of SC can improve the readiness of the uterus by aiding in the growth of spiral arteries and augmenting the flow of arterial blood within the uterine region. Fourth, SC may increase natural killer cell activity in addition to the role of SC in promoting endometrial growth facilitation[51]. The fifth point is that SC can trigger the angiogenic reactions of the vascular endothelial growth factor. This involvement in the formation of new blood vessels and heightened vascular penetrability in the middle secretory stage is fundamental to the achievement of satisfactory implantation[52].

In our current systematic review and meta-analysis, we examined 28 RCTs involving 3.426 women with subfertility. Among these participants, 1.702 were assigned to receive the intervention, whereas 1.724 were placed in the control group. All individuals underwent ART. Based on the collective findings, it is evident that the use of SC, either alone or in conjunction with other active treatments such as CC, estradiol valerate, or letrozole, can lead to higher chemical pregnancy rate, clinical pregnancy rate, and endometrial thickness compared with the control group. However, it is essential to approach these findings with concern because the quality of the RCTs included was not up to par. When subgroup analysis was conducted, it was found that women with RIF experienced the most significant increase in chemical and clinical pregnancy rates compared with patients with other infertility causes. Various RCTs included infertile patients with unexplained infertility or narrow endometrium. Considering the various sources of infertility investigated in the eligible RCTs and the inadequate number of available RCTs, it was impossible to conduct a proper subgroup analysis. Therefore, it is important to be cautious when interpreting the results of this analysis.

Furthermore, due to inadequate information on the methodologies used in the included studies, we could not perform subgroup analysis considering various potential factors contributing to heterogeneity, such as the specific dose of SC, the stage of embryos during transfer, or the type of protocol employed[24-26]. Moreover, although we did identify a noteworthy distinction between women who were administered SC and those who received a placebo, the available evidence of endometrial thickening was of extremely poor quality, exhibiting imprecise results and significant heterogeneity. Lastly, none of the RCTs included in our analysis offered data on live birth rates or side effects, resulting in a lack of conclusive evidence regarding differences between the groups in these aspects.

Prior to our investigation, many systematic reviews and meta-analyses had examined the effectiveness of various treatments (such as gonadotropin-releasing hormone agonist[53], aspirin[12], human chorionic gonadotrophin[54], and a mixture of alpha-tocopherol plus pentoxifylline[55] in preparing the endometrium for patients experiencing ART. Nonetheless, the existing evidence was insufficient to establish a specific procedure for preparation of the endometrium. We came across only one meta-analysis that assessed the clinical utility of vasodilation-promoting agents in patients undergoing ART. This analysis spanned six data sources and encompassed 15 studies and 1.326 patients. All meta-analyzed RCTs contrasted a vasodilator with a control group comprising no treatment or placebo. Three of the RCTs examined the clinical utility of SC. Two of the RCTs judged the efficacy of monotherapy SC compared with placebo, while the other RCT assessed the blend of SC and estradiol valerate compared with no treatment. The meta-analysis of the two articles comparing SC alone with placebo indicated no discernible differences between the two treatments. Likewise, an investigation comparing the blend of SC and estradiol valerate indicated no significant changes between the two interventions[20]. In addition to these three

RCTs, we identified 25 additional RCTs that evaluated the efficacy of SC either alone or in conjunction with other active therapies. The most extensive RCT to date, conducted in Egypt, examined the effectiveness of combining SC and CC compared with CC alone and was well-executed.

At Ain-Shams University Women's Hospital, an RCT was conducted involving 850 women diagnosed with PCOS who had previously experienced failure with CC. The study compared two groups, one receiving SC treatment and the other receiving CC treatment. The SC group demonstrated a greater thickness of the endometrial lining and had a higher likelihood of achieving a clinical pregnancy than the CC group[29].

The methodological quality and similarity of the studies included have a sizable influence on the reliability of the meta-analysis results. In our study, the quality of the trials included was comparatively poor, but there was little variation in the analysis of clinical and chemical pregnancy rates. However, we found high heterogeneity in the analysis of endometrial thickness. Although we did not find any statistical heterogeneity in the main outcomes across the trials, we cannot disregard the potential impact of clinical heterogeneity on the study findings. While most RCTs examined in the review had clearly defined criteria for including and excluding participants, a small number of RCTs failed to specify their precise criteria. In addition, the RCTs lacked consistent or unreported information about the primary source of infertility and the history of RIF or repeated abortion among the women included in the research. There was also variation across the RCTs with respect to the methods used for luteal stage stimulation, endometrial setup, and ovarian hyperstimulation regimens, with some studies failing to report these specific details. Moreover, there were notable variations in the dosage of SC, a crucial element that significantly impacts the efficacy of the given medication, across the various studies. Unfortunately, most studies did not report miscarriage rates, drug-related adverse events, or pregnancy outcomes, thereby limiting our understanding of these important factors.

Regarding the effects of SC on frozen-thawed embryo transfer (FET) cycles in the setting of ART, our results from included studies advocate that SC may positively affect endometrial receptivity, potentially enhancing blood flow to the uterus and improving the implantation rates of embryos during FET[19]. The medication is thought to exert influence by relaxing uterine smooth muscles and increasing blood flow, which could contribute to a more favorable environment for embryo implantation[56]. However, research on this topic is ongoing, and while some studies have shown promising results, further investigation is needed to establish the optimal dosage, timing, and overall efficacy of SC in the context of FET cycles[57].

There were various shortcomings in our review. First, the majority of the RCTs we analyzed depicted inadequate methodological quality and harbored a small number of patients. These factors can affect the reliability of the studies. Second, there was significant variability in some results, especially with regard to endometrial thickness. However, we did not perform subgroup analyses to investigate the potential causes of this variability, such as the specific dose of SC, stage of embryo transfer, or type of protocol used. This was because the analyzed studies did not offer sufficient detailed data about their methods.

Conclusion

The present systematic review and meta-analysis revealed that luteal administration of SC, whether orally or vaginally, either alone or as an adjuvant therapy, may enhance endometrial thickness and clinical pregnancy rate in women experiencing ART. Considering the methodological limitations of the analyzed RCTs, this evidence does not yet endorse the routine use of SC in clinical practice. To establish its efficacy and safety more conclusively, future high-quality RCTs with larger sample sizes are necessary. Future RCTs should prioritize different elements, including processing methods, embryo stage, embryo quality, dosage, administration timing, and the composition of the control group. These studies aim to pinpoint particular patient groups that would derive the greatest advantages from this drug administration and establish the ideal dose, timing, and type of SC that would produce the most favorable outcomes while minimizing potential adverse events.

Ethics

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

Concept: S.B., A.A-Z., Design: S.B., A.A-Z., Data Collection or Processing: S.B., M.A., M.S.A., H.T.S., W.H.A., E.B., A.Y.A., I.A.B., A.A., O.A., A.A-Z., Analysis or Interpretation: S.B., M.A., O.A., A.A-Z., Literature Search: M.A., M.S.A., H.T.S., W.H.A., E.B., A.Y.A., I.A.B., A.A., O.A., Writing: S.B., A.A-Z.

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Supplement **Table 1.** Detailed search strategy

Domains	Descriptors
Population	Reproductive Techniques, Assisted OR Assisted Reproductive Technique OR Reproductive Technique, Assisted OR Technique, Assisted Reproductive OR Assisted Reproductive Technics OR Assisted Reproductive Technology OR Assisted Reproductive Technologies OR Reproductive Technologies, Assisted OR Fertilization in Vitro OR In Vitro Fertilization OR In Vitro Fertilizations OR IVF OR ICSI OR Sperm Injections, Intracytoplasmic OR Injection, Intracytoplasmic Sperm OR Intracytoplasmic Sperm Injection OR Injections, Intracytoplasmic Sperm OR in-vitro fertilization OR Recurrent in Vitro Fertilization failure OR recurrent in-vitro fertilization failure OR Recurrent IVF failure OR recurrent implantation failure OR Recurrent failure of implantation OR Recurrent failure of in vitro fertilization OR RIF OR Repeated implantation failure OR failed cycle OR implantation failure OR recurrent failure to implant OR repeat failure to implant OR recurrent failed implantation OR repeat failed implantation OR recurrent reproductive failure OR repeat reproductive failure OR poor implantation
Intervention	Sildenafil Citrate OR Citrate, Sildenafil OR Sildenafil OR Homosildenafil OR Hydroxyhomosildenafil OR Revatio OR Viagra OR Acetildenafil OR Sildenafil Lactate OR Lactate, Sildenafil OR Sildenafil Nitrate OR Nitrate, Sildenafil OR Desmethyl Sildenafil OR Sildenafil, Desmethyl OR Desmethylsildenafil
Design	Randomized controlled trial OR controlled clinical trial OR randomized controlled trials OR random allocation OR double-blind method OR single blind method OR clinical trial OR clinical trials OR placebos OR placebo OR random

PubMed
Number of localized studies: 99
Limits: Clinical Trial, Humans

	Descriptors	Number of studies reached
#1	(((((((((((((((((((((((((((((((((((((“reproductive techniques, assisted”[MeSH Terms] OR (“reproductive”[All Fields] AND “techniques”[All Fields] AND “assisted”[All Fields]) OR “assisted reproductive techniques”[All Fields] OR (“reproductive”[All Fields] AND “techniques”[All Fields] AND “assisted”[All Fields]) OR “reproductive techniques, assisted”[All Fields]) OR (“reproductive techniques, assisted”[MeSH Terms] OR (“reproductive”[All Fields] AND “techniques”[All Fields] AND “assisted”[All Fields]) OR “assisted reproductive techniques”[All Fields] OR (“assisted”[All Fields] AND “reproductive”[All Fields] AND “technique”[All Fields]) OR “assisted reproductive technique”[All Fields])) OR (“reproductive techniques, assisted”[MeSH Terms] OR (“reproductive”[All Fields] AND “techniques”[All Fields] AND “assisted”[All Fields]) OR “assisted reproductive techniques”[All Fields] OR (“reproductive”[All Fields] AND “technique”[All Fields] AND “assisted”[All Fields]))) OR (“reproductive techniques, assisted”[MeSH Terms] OR (“reproductive”[All Fields] AND “techniques”[All Fields] AND “assisted”[All Fields]) OR “assisted reproductive techniques”[All Fields] OR (“technique”[All Fields] AND “assisted”[All Fields] AND “reproductive”[All Fields]))) OR (“reproductive techniques, assisted”[MeSH Terms] OR (“reproductive”[All Fields] AND “techniques”[All Fields] AND “assisted”[All Fields]) OR “assisted reproductive techniques”[All Fields] OR (“assisted”[All Fields] AND “reproductive”[All Fields] AND “technics”[All Fields]) OR “assisted reproductive technics”[All Fields])) OR (“reproductive techniques, assisted”[MeSH Terms] OR (“reproductive”[All Fields] AND “techniques”[All Fields] AND “assisted”[All Fields]) OR “assisted reproductive techniques”[All Fields] OR (“assisted”[All Fields] AND “reproductive”[All Fields] AND “technology”[All Fields]) OR “assisted reproductive technology”[All Fields])) OR (“reproductive techniques, assisted”[MeSH Terms] OR (“reproductive”[All Fields] AND “techniques”[All Fields] AND “assisted”[All Fields]) OR “assisted reproductive techniques”[All Fields] OR (“assisted”[All Fields] AND “reproductive”[All Fields] AND “technologies”[All Fields]) OR “assisted reproductive technologies”[All Fields])) OR (“reproductive techniques, assisted”[MeSH Terms] OR (“reproductive”[All Fields] AND “techniques”[All Fields] AND “assisted”[All Fields]) OR “assisted reproductive techniques”[All Fields] OR (“reproductive”[All Fields] AND “technologies”[All Fields] AND “assisted”[All Fields]))) OR (“fertilisation in vitro”[All Fields] OR “fertilization in vitro”[MeSH Terms] OR (“fertilization”[All Fields] AND “vitro”[All Fields]) OR “fertilization in vitro”[All Fields])) OR (“in vitro fertilisation”[All Fields] OR “fertilization in vitro”[MeSH Terms] OR (“fertilization”[All Fields] AND “vitro”[All Fields]) OR “fertilization in vitro”[All Fields] OR (“vitro”[All Fields] AND “fertilization”[All Fields]) OR “in vitro fertilization”[All Fields])) OR (“fertilization in vitro”[MeSH Terms] OR (“fertilization”[All Fields] AND “vitro”[All Fields]) OR “fertilization in vitro”[All Fields] OR (“vitro”[All Fields] AND “fertilizations”[All Fields]) OR “in vitro fertilizations”[All Fields])) OR (“sperm injections, intracytoplasmic”[MeSH Terms] OR (“sperm”[All Fields] AND “injections”[All Fields] AND “intracytoplasmic”[All Fields]) OR “intracytoplasmic sperm injections”[All Fields] OR (“sperm”[All Fields] AND “injections”[All Fields] AND “intracytoplasmic”[All Fields]) OR “sperm injections, intracytoplasmic”[All Fields])) OR (“sperm injections, intracytoplasmic”[MeSH Terms] OR (“sperm”[All Fields] AND “injections”[All Fields] AND “intracytoplasmic”[All Fields]) OR “intracytoplasmic sperm injections”[All Fields] OR (“injection”[All Fields] AND “intracytoplasmic”[All Fields] AND “sperm”[All Fields]))) OR (“sperm injections, intracytoplasmic”[MeSH Terms] OR (“sperm”[All Fields] AND “injections”[All Fields] AND “intracytoplasmic”[All Fields]) OR “intracytoplasmic sperm injections”[All Fields] OR (“intracytoplasmic”[All Fields] AND “sperm”[All Fields] AND “injection”[All Fields]) OR “intracytoplasmic sperm injection”[All Fields])) OR (“sperm injections, intracytoplasmic”[MeSH Terms] OR (“sperm”[All Fields] AND “injections”[All Fields] AND “intracytoplasmic”[All Fields]) OR “intracytoplasmic sperm injections”[All Fields] OR (“injections”[All Fields] AND “intracytoplasmic”[All Fields] AND “sperm”[All Fields]))) OR (“J In Vitro Fert Embryo Transf”[Journal] OR “ivf”[All Fields])) OR (“in vitro fertilisation”[All Fields] OR “fertilization in vitro”[MeSH Terms] OR (“fertilization”[All Fields] AND “vitro”[All Fields]) OR “fertilization in vitro”[All Fields] OR (“vitro”[All Fields] AND “fertilization”[All Fields]) OR “in vitro fertilization”[All Fields])) OR (“in vitro fertilisation”[All Fields] OR “fertilization in vitro”[MeSH Terms] OR (“fertilization”[All Fields] AND “vitro”[All Fields]) OR “fertilization in vitro”[All Fields] OR (“vitro”[All Fields] AND “fertilization”[All Fields]) OR “in vitro fertilization”[All Fields])) OR (“sperm injections, intracytoplasmic”[MeSH Terms] OR (“sperm”[All Fields] AND “injections”[All Fields] AND “intracytoplasmic”[All Fields]) OR “intracytoplasmic sperm injections”[All Fields] OR “icsi”[All Fields])) OR (“sperm injections, intracytoplasmic”[MeSH Terms] OR (“sperm”[All Fields] AND “injections”[All Fields] AND “intracytoplasmic”[All Fields]) OR “intracytoplasmic sperm injections”[All Fields] OR (“intracytoplasmic”[All Fields] AND “sperm”[All Fields] AND “injection”[All Fields]) OR “intracytoplasmic sperm injection”[All Fields])) OR (“in vitro fertilisation”[All Fields] OR “fertilization in vitro”[MeSH Terms] OR (“fertilization”[All Fields] AND “vitro”[All Fields]) OR “fertilization in vitro”[All Fields] OR (“vitro”[All Fields] AND “fertilization”[All Fields]) OR “in vitro fertilization”[All Fields])) OR (Recurrent[All Fields] AND (“in vitro fertilisation”[All Fields] OR “fertilization in vitro”[MeSH Terms] OR (“fertilization”[All Fields] AND “vitro”[All Fields]) OR “fertilization in vitro”[All Fields] OR (“vitro”[All Fields] AND “fertilization”[All Fields]) OR “in vitro fertilization”[All Fields]) AND failure[All Fields])) OR (recurrent[All Fields] AND (“in vitro fertilisation”[All Fields] OR “fertilization in vitro”[MeSH Terms] OR (“fertilization”[All Fields] AND “vitro”[All Fields]) OR “fertilization in vitro”[All Fields] OR (“vitro”[All Fields] AND “fertilization”[All Fields]) OR “in vitro fertilization”[All Fields]) AND failure[All Fields])) OR (Recurrent[All Fields] AND (“J In Vitro Fert Embryo Transf”[Journal] OR “ivf”[All Fields]) AND failure[All Fields])) OR (recurrent[All Fields] AND (“embryo implantation”[MeSH Terms] OR (“embryo”[All Fields] AND “implantation”[All Fields]) OR “embryo implantation”[All Fields] OR “implantation”[All Fields]) AND failure[All Fields])) OR (Recurrent[All Fields] AND failure[All Fields] AND (“embryo implantation”[MeSH Terms] OR (“embryo”[All Fields] AND “implantation”[All Fields]) OR “embryo implantation”[All Fields] OR “implantation”[All Fields]))) OR (Recurrent[All Fields] AND failure[All Fields] AND (“in vitro fertilisation”[All Fields] OR “fertilization in vitro”[MeSH Terms] OR (“fertilization”[All Fields] AND “vitro”[All Fields]) OR “fertilization in vitro”[All Fields] OR (“vitro”[All Fields] AND “fertilization”[All Fields]) OR “in vitro fertilization”[All Fields]))) OR (“Rev Infect”[Journal] OR “rif”[All Fields])) OR (Repeated[All Fields] AND (“embryo implantation”[MeSH Terms] OR (“embryo”[All Fields] AND “implantation”[All Fields]) OR “embryo implantation”[All Fields] OR “implantation”[All Fields]) AND failure[All Fields])) OR (failed[All Fields] AND cycle[All Fields])) OR ((“embryo implantation”[MeSH Terms] OR (“embryo”[All Fields] AND “implantation”[All Fields]) OR “embryo implantation”[All Fields] OR “implantation”[All Fields]) AND failure[All Fields])) OR (recurrent[All Fields] AND failure[All Fields] AND implant[All Fields])) OR (repeat[All Fields] AND failure[All Fields] AND implant[All Fields])) OR (recurrent[All Fields] AND failed[All Fields] AND (“embryo implantation”[MeSH Terms] OR (“embryo”[All Fields] AND “implantation”[All Fields]) OR “embryo implantation”[All Fields] OR “implantation”[All Fields]))) OR (repeat[All Fields] AND failed[All Fields] AND (“embryo implantation”[MeSH Terms] OR (“embryo”[All Fields] AND “implantation”[All Fields]) OR “embryo implantation”[All Fields] OR “implantation”[All Fields]))) OR (repeat[All Fields] AND (“reproduction”[MeSH Terms] OR “reproduction”[All Fields] OR “reproductive”[All Fields]) AND failure[All Fields])) OR ((“poverty”[MeSH Terms] OR “poverty”[All Fields] OR “poor”[All Fields]) AND (“embryo implantation”[MeSH Terms] OR (“embryo”[All Fields] AND “implantation”[All Fields]) OR “embryo implantation”[All Fields] OR “implantation”[All Fields]))	176,256

PubMed
Number of localized studies: 99
Limits: Clinical Trial, Humans

	Descriptors	Number of studies reached
#2	((((((((((("sildenafil citrate"[MeSH Terms] OR ("sildenafil"[All Fields] AND "citrate"[All Fields]) OR "sildenafil citrate"[All Fields]) OR ("sildenafil citrate"[MeSH Terms] OR ("sildenafil"[All Fields] AND "citrate"[All Fields]) OR "sildenafil citrate"[All Fields] OR ("citrate"[All Fields] AND "sildenafil"[All Fields]))) OR ("sildenafil citrate"[MeSH Terms] OR ("sildenafil"[All Fields] AND "citrate"[All Fields]) OR "sildenafil citrate"[All Fields] OR "sildenafil"[All Fields])) OR ("sildenafil citrate"[MeSH Terms] OR ("sildenafil"[All Fields] AND "citrate"[All Fields]) OR "sildenafil citrate"[All Fields] OR "homosildenafil"[All Fields])) OR ("sildenafil citrate"[MeSH Terms] OR ("sildenafil"[All Fields] AND "citrate"[All Fields]) OR "sildenafil citrate"[All Fields] OR "hydroxyhomosildenafil"[All Fields])) OR ("sildenafil citrate"[MeSH Terms] OR ("sildenafil"[All Fields] AND "citrate"[All Fields]) OR "sildenafil citrate"[All Fields] OR "revatio"[All Fields])) OR ("sildenafil citrate"[MeSH Terms] OR ("sildenafil"[All Fields] AND "citrate"[All Fields]) OR "sildenafil citrate"[All Fields] OR "acetildenafil"[All Fields])) OR ("sildenafil citrate"[MeSH Terms] OR ("sildenafil"[All Fields] AND "citrate"[All Fields]) OR "sildenafil citrate"[All Fields] OR ("sildenafil"[All Fields] AND "lactate"[All Fields]) OR "sildenafil lactate"[All Fields])) OR ("sildenafil citrate"[MeSH Terms] OR ("sildenafil"[All Fields] AND "citrate"[All Fields]) OR "sildenafil citrate"[All Fields] OR ("lactate"[All Fields] AND "sildenafil"[All Fields]))) OR ("sildenafil citrate"[MeSH Terms] OR ("sildenafil"[All Fields] AND "citrate"[All Fields]) OR "sildenafil citrate"[All Fields] OR ("nitrate"[All Fields] AND "sildenafil"[All Fields]))) OR ("sildenafil citrate"[MeSH Terms] OR ("sildenafil"[All Fields] AND "citrate"[All Fields]) OR "sildenafil citrate"[All Fields] OR ("desmethyl"[All Fields] AND "sildenafil"[All Fields]) OR "desmethyl sildenafil"[All Fields])) OR ("sildenafil citrate"[MeSH Terms] OR ("sildenafil"[All Fields] AND "citrate"[All Fields]) OR "sildenafil citrate"[All Fields] OR ("sildenafil"[All Fields] AND "desmethyl"[All Fields]))) OR ("sildenafil citrate"[MeSH Terms] OR ("sildenafil"[All Fields] AND "citrate"[All Fields]) OR "sildenafil citrate"[All Fields] OR "desmethylsildenafil"[All Fields])	8,513
#3	**#1** AND **#2**	99

Web of Science
Number of localized studies: 196
Limits: no limit

	Descriptors	Number of studies reached
#1	(Reproductive Techniques, Assisted) OR TOPIC: (Assisted Reproductive Technique) OR TOPIC: (Reproductive Technique, Assisted) OR TOPIC: (Technique, Assisted Reproductive) OR TOPIC: (Assisted Reproductive Technics) OR TOPIC: (Assisted Reproductive Technology) OR TOPIC: (Assisted Reproductive Technologies) OR TOPIC: (Reproductive Technologies, Assisted) OR TOPIC: (Fertilization in Vitro) OR TOPIC: (In Vitro Fertilization) OR TOPIC: (In Vitro Fertilizations) OR TOPIC: (IVF) OR TOPIC: (ICSI) OR TOPIC: (Sperm Injections, Intracytoplasmic) OR TOPIC: (Injection, Intracytoplasmic Sperm) OR TOPIC: (Intracytoplasmic Sperm Injection) OR TOPIC: (Injections, Intracytoplasmic Sperm) OR TOPIC: (in-vitro fertilization) OR TOPIC: (Recurrent in Vitro Fertilization failure) OR TOPIC: (recurrent in-vitro fertilization failure) OR TOPIC: (Recurrent IVF failure) OR TOPIC: (recurrent implantation failure) OR TOPIC: (Recurrent failure of implantation) OR TOPIC: (Recurrent failure of in vitro fertilization) OR TOPIC: (RIF) OR (Repeated implantation failure) OR TOPIC: (failed cycle) OR TOPIC: (implantation failure) OR TOPIC: (recurrent failure to implant) OR TOPIC: (repeat failure to implant) OR TOPIC: (recurrent failed implantation) OR TOPIC: (repeat failed implantation) OR TOPIC: (recurrent reproductive failure) OR TOPIC: (repeat reproductive failure) OR TOPIC: (poor implantation)	162,807
#2	TOPIC: (Sildenafil Citrate) OR TOPIC: (Citrate, Sildenafil) OR TOPIC: (Sildenafil) OR TOPIC: (Homosildenafil) OR TOPIC: (Hydroxyhomosildenafil) OR TOPIC: (Revatio) OR TOPIC: (Viagra) OR TOPIC: (Acetildenafil) OR TOPIC: (Sildenafil Lactate) OR TOPIC: (Lactate, Sildenafil) OR TOPIC: (Sildenafil Nitrate) OR TOPIC: (Nitrate, Sildenafil) OR TOPIC: (Desmethyl Sildenafil) OR TOPIC: (Sildenafil, Desmethyl) OR TOPIC: (Desmethylsildenafil)	12,869
#3	#1 AND #2	196

Scopus
Number of localized studies: 336

	Descriptors	Number of studies reached
#1	((TITLE-ABS-KEY (reproductive AND techniques, AND assisted) OR TITLE-ABS-KEY (assisted AND reproductive AND technique) OR TITLE-ABS-KEY (reproductive AND technique, AND assisted) OR TITLE-ABS-KEY (technique, AND assisted AND reproductive) OR TITLE-ABS-KEY (assisted AND reproductive AND technics) OR TITLE-ABS-KEY (assisted AND reproductive AND technology) OR TITLE-ABS-KEY (assisted AND reproductive AND technologies) OR TITLE-ABS-KEY (reproductive AND technologies, AND assisted) OR TITLE-ABS-KEY (fertilization AND in AND vitro) OR TITLE-ABS-KEY (in AND vitro AND fertilization) OR TITLE-ABS-KEY (in AND vitro AND fertilizations) OR TITLE-ABS-KEY (ivf))) OR ((TITLE-ABS-KEY (icsi) OR TITLE-ABS-KEY (sperm AND injections, AND intracytoplasmic) OR TITLE-ABS-KEY (injection, AND intracytoplasmic AND sperm) OR TITLE-ABS-KEY (intracytoplasmic AND sperm AND injection) OR TITLE-ABS-KEY (injections, AND intracytoplasmic AND sperm) OR TITLE-ABS-KEY (in-vitro AND fertilization) OR TITLE-ABS-KEY (assisted AND reproductive AND technologies) OR TITLE-ABS-KEY (recurrent AND in AND vitro AND fertilization AND failure) OR TITLE-ABS-KEY (recurrent AND in-vitro AND fertilization AND failure) OR TITLE-ABS-KEY (recurrent AND ivf AND failure) OR TITLE-ABS-KEY (recurrent AND implantation AND failure) OR TITLE-ABS-KEY (recurrent AND failure AND of AND implantation) OR TITLE-ABS-KEY (recurrent AND failure AND of AND in AND vitro AND fertilization) OR TITLE-ABS-KEY (rif) OR TITLE-ABS-KEY (repeated AND implantation AND failure) OR TITLE-ABS-KEY (failed AND cycle) OR TITLE-ABS-KEY (implantation AND failure) OR TITLE-ABS-KEY (recurrent AND failure AND to AND implant) OR TITLE-ABS-KEY (repeat AND failure AND to AND implant) OR TITLE-ABS-KEY (recurrent AND failed AND implantation) OR TITLE-ABS-KEY (repeat AND failed AND implantation) OR TITLE-ABS-KEY (recurrent AND reproductive AND failure) OR TITLE-ABS-KEY (repeat AND reproductive AND failure) OR TITLE-ABS-KEY (poor AND implantation)))	202,497
#2	(TITLE-ABS-KEY (sildenafil AND citrate) OR TITLE-ABS-KEY (citrate, AND sildenafil) OR TITLE-ABS-KEY (sildenafil) OR TITLE-ABS-KEY (homosildenafil) OR TITLE-ABS-KEY (hydroxyhomosildenafil) OR TITLE-ABS-KEY (revatio) OR TITLE-ABS-KEY (viagra) OR TITLE-ABS-KEY (acetildenafil) OR TITLE-ABS-KEY (sildenafil AND lactate) OR TITLE-ABS-KEY (lactate, AND sildenafil) OR TITLE-ABS-KEY (sildenafil AND nitrate) OR TITLE-ABS-KEY (nitrate, AND sildenafil) OR TITLE-ABS-KEY (desmethyl AND sildenafil) OR TITLE-ABS-KEY (sildenafil, AND desmethyl) OR TITLE-ABS-KEY (desmethylsildenafil))	22,447
#3	#1 AND #2	336

Cochrane
Number of localized studies: 88
Limits: No limits

	Descriptors	Number of studies reached
#1	Reproductive Techniques, Assisted OR Assisted Reproductive Technique OR Reproductive Technique, Assisted OR Technique, Assisted Reproductive OR Assisted Reproductive Technics OR Assisted Reproductive Technology OR Assisted Reproductive Technologies OR Reproductive Technologies, Assisted OR Fertilization in Vitro OR In Vitro Fertilization OR In Vitro Fertilizations OR IVF OR ICSI OR Sperm Injections, Intracytoplasmic OR Injection, Intracytoplasmic Sperm OR Intracytoplasmic Sperm Injection OR Injections, Intracytoplasmic Sperm OR in-vitro fertilization OR Recurrent in Vitro Fertilization failure OR recurrent in-vitro fertilization failure OR Recurrent IVF failure OR recurrent implantation failure OR Recurrent failure of implantation OR Recurrent failure of in vitro fertilization OR RIF OR Repeated implantation failure OR failed cycle OR implantation failure OR recurrent failure to implant OR repeat failure to implant OR recurrent failed implantation OR repeat failed implantation OR recurrent reproductive failure OR repeat reproductive failure OR poor implantation in Title Abstract Keyword - (Word variations have been searched)	37,144
#2	Sildenafil Citrate OR Citrate, Sildenafil OR Sildenafil OR Homosildenafil OR Hydroxyhomosildenafil OR Revatio OR Viagra OR Acetildenafil OR Sildenafil Lactate OR Lactate, Sildenafil OR Sildenafil Nitrate OR Nitrate, Sildenafil OR Desmethyl Sildenafil OR Sildenafil, Desmethyl OR Desmethylsildenafil	2,274
#3	#1 AND #2	88

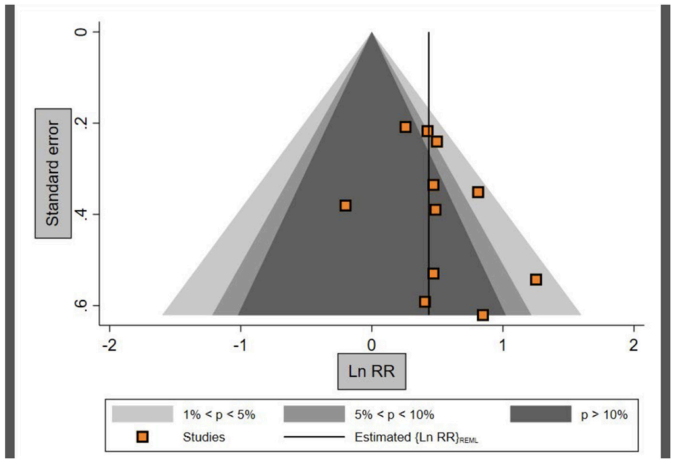
Embase
Number of localized studies: 398
Limits: No limits

	Descriptors	Number of studies reached
#1	reproductive AND techniques, AND assisted OR (assisted AND reproductive AND technique) OR (reproductive AND technique, AND assisted) OR (technique, AND assisted AND reproductive) OR (assisted AND reproductive AND technics) OR (assisted AND reproductive AND technology) OR (assisted AND reproductive AND technologies) OR (reproductive AND technologies, AND assisted) OR (fertilization AND in AND vitro) OR (in AND vitro AND fertilization) OR (in AND vitro AND fertilizations) OR ivf OR icsi OR (sperm AND injections, AND intracytoplasmic) OR (injection, AND intracytoplasmic AND sperm) OR (intracytoplasmic AND sperm AND injection) OR (injections, AND intracytoplasmic AND sperm) OR ('in vitro' AND fertilization) OR (recurrent AND in AND vitro AND fertilization AND failure) OR (recurrent AND 'in vitro' AND fertilization AND failure) OR (recurrent AND ivf AND failure) OR (recurrent AND implantation AND failure) OR (recurrent AND failure AND of AND implantation) OR (recurrent AND failure AND of AND in AND vitro AND fertilization) OR rif OR (repeated AND implantation AND failure) OR (failed AND cycle) OR (implantation AND failure) OR (recurrent AND failure AND to AND implant) OR (repeat AND failure AND to AND implant) OR (recurrent AND failed AND implantation) OR (repeat AND failed AND implantation) OR (recurrent AND reproductive AND failure) OR (repeat AND reproductive AND failure) OR (poor AND implantation)	196,523
#2	sildenafil AND citrate OR (citrate, AND sildenafil) OR sildenafil OR homosildenafil OR hydroxyhomosildenafil OR revatio OR viagra OR acetildenafil OR (sildenafil AND lactate) OR (lactate, AND sildenafil) OR (sildenafil AND nitrate) OR (nitrate, AND sildenafil) OR (desmethyl AND sildenafil) OR (sildenafil, AND desmethyl) OR desmethylsildenafil	24,812
#3	#1 AND #2	398

Records from electronic databases: 1,117
Records from other resources: 69
Total with duplicates: 1,184
Duplicates: 249
Final records for review: 535

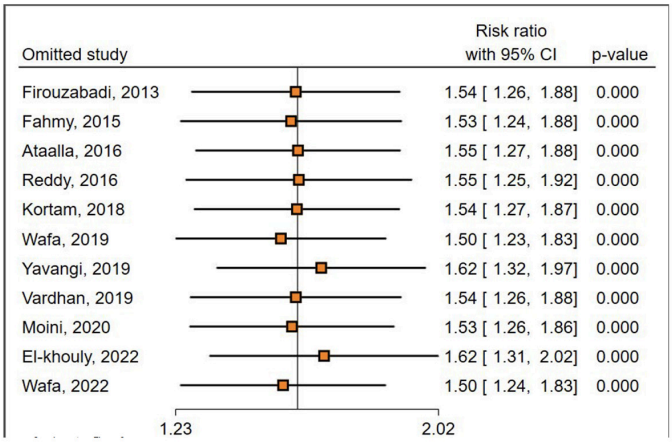
Supplement Table 2. Assessment of the risk of bias in the included trials

Author, year	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
Moini et al.[17] 2020	-	+	?	?	+	-	-
El-Sayed et al.[25] 2017	?	+	_	_	?	?	_
Tehraninejad et al.[43] 2018	?	+	+	_	?	_	_
Ataalla et al.[26] 2018	_	+	_	_	_	_	_
Wafa et al.[24] 2019	_	+	_	_	_	_	_
Kortam et al.[27] 2018	_	_	+	?	+	?	_
Reddy et al.[38] 2016	?	+	+	_	+	?	_
AbdelKader Fahmy et al.[28] 2015	_	_	+	?	+	?	_
Ashoush and Abdelshafy[29] 2019	_	_	_	_	_	_	_
Aboelroose et al.[30] 2020	_	_	_	_	_	_	_
Yavangi et al.[44] 2019	_	_	_	?	_	_	_
Vardhan et al.[39] 2019	?	+	+	?	_	?	_
Mangal and Mehirishi[40] 2019	?	+	+	?	_	?	_
Dehghani Firouzabadi et al.[19] 2019	_	?	?	_	_	_	_
Alieva et al.[48] 2012	?	?	?	?	?	?	?
Kim et al.[49] 2010	?	?	?	_	_	?	_
Abdullah et al.[45] 2021	?	+	+	+	_	?	?
Belapurkar et al.[42] 2022	_	?	+	+	_	_	_
Mohammed et al.[46] 2023	?	+	+	+	_	?	?
Dawood et al.[47] 2020	?	+	+	+	_	?	?
Mohamed et al.[18] 2022	_	?	+	+	_	_	_
Gupta et al.[41] 2021	?	+	+	+	?	?	?
Abbas et al.[32] 2021	_	?	+	+	_	_	_
ELkhouly et al.[33] 2022	_	?	+	+	_	_	?
El-Asbaa et al.[34] 2021	_	?	+	+	_	_	?
Abdel Hamid et al.[35] 2021	_	_	+	+	_	?	?
Wafa et al.[24] 2022	?	+	_	_	?	?	_
El-ghany et al.[37] 2022	?	+	_	_	?	?	_



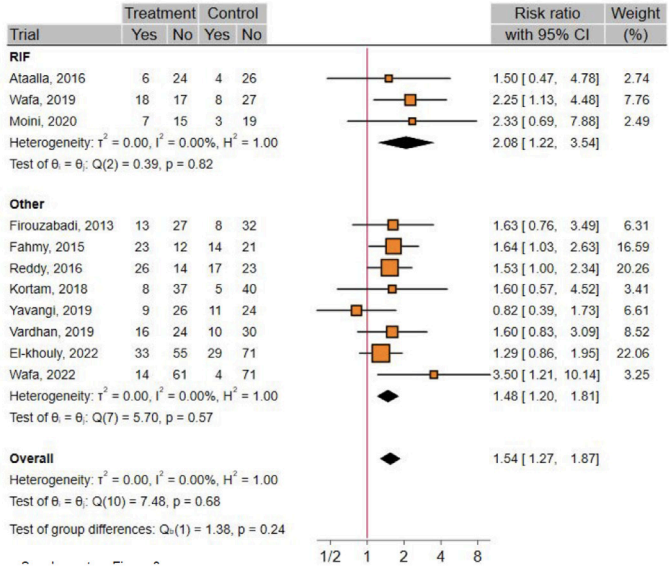
Supplement Figure 1. Funnel plot showing publication bias in trials that evaluated the risk of chemical pregnancy in women who received treatment versus control

RR: Risk ratio

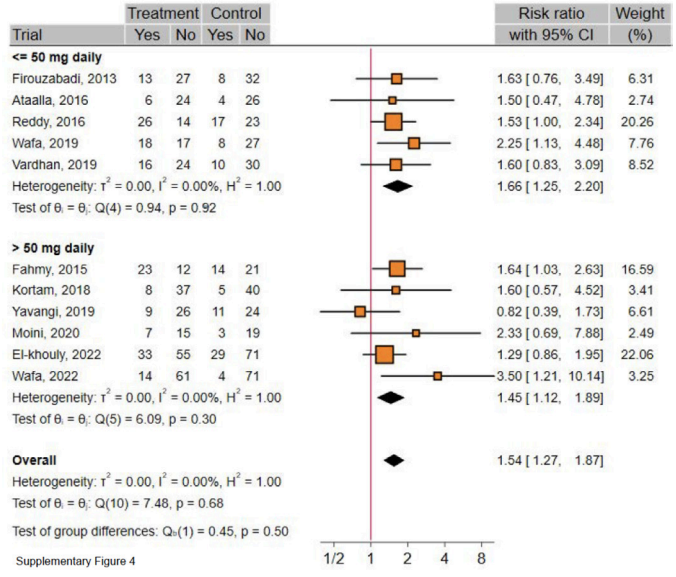


Supplement Figure 2. Leave-one-out plot showing the effect of each study on pooled estimate in subset of trials that evaluated the risk of chemical pregnancy in women who received treatment versus control

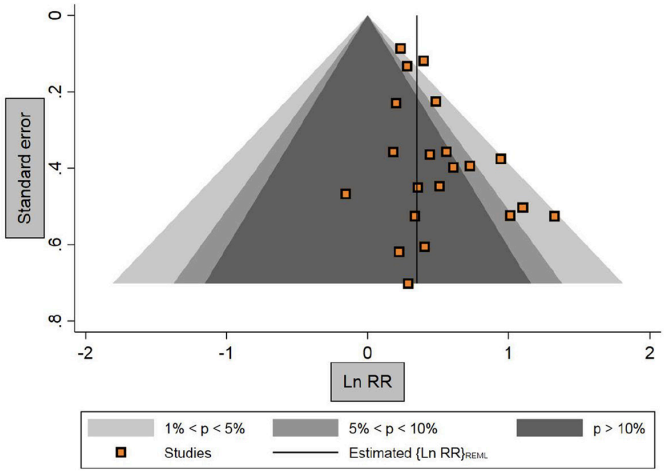
CI: Confidence interval



Supplement Figure 3. Forest plot showing individual and combined effect size estimates and 95% confidence intervals (CIs) in trials that evaluated the risk of chemical pregnancy in women who received intervention versus control regarding population type

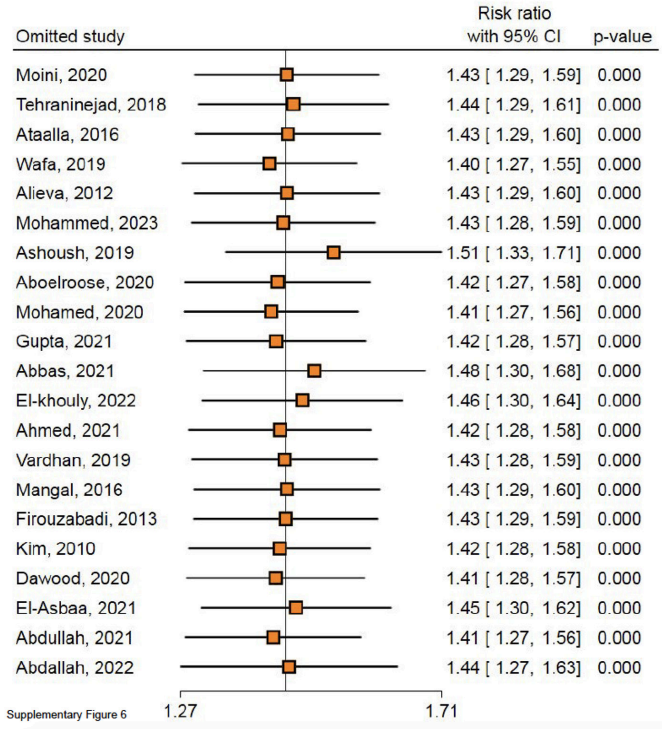


Supplement Figure 4. Forest plot showing individual and combined effect size estimates and 95% confidence intervals (CIs) in trials that evaluated the risk of chemical pregnancy in women who received intervention versus control regarding dose of sildenafil citrate



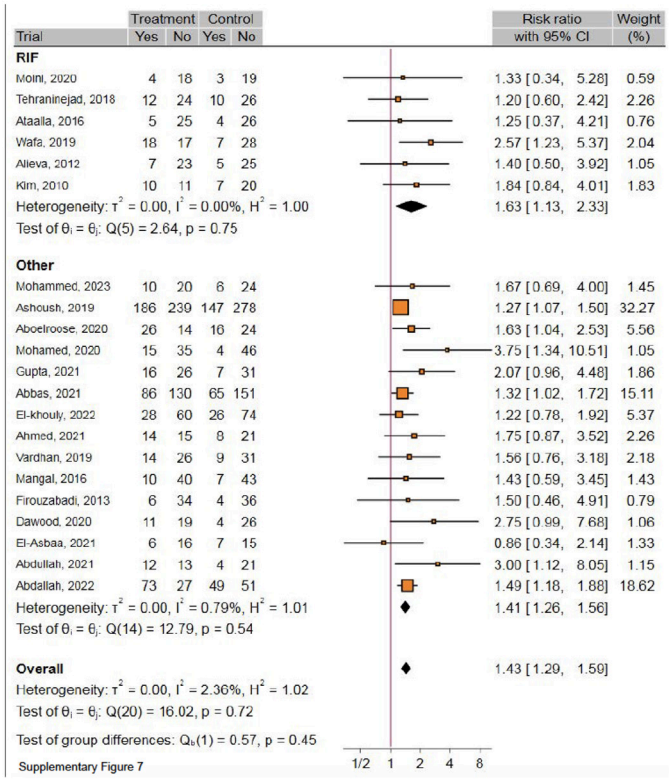
Supplement Figure 5. Funnel plot showing publication bias in trials that evaluated the risk of clinical pregnancy in women who received treatment versus control

RR: Risk ratio

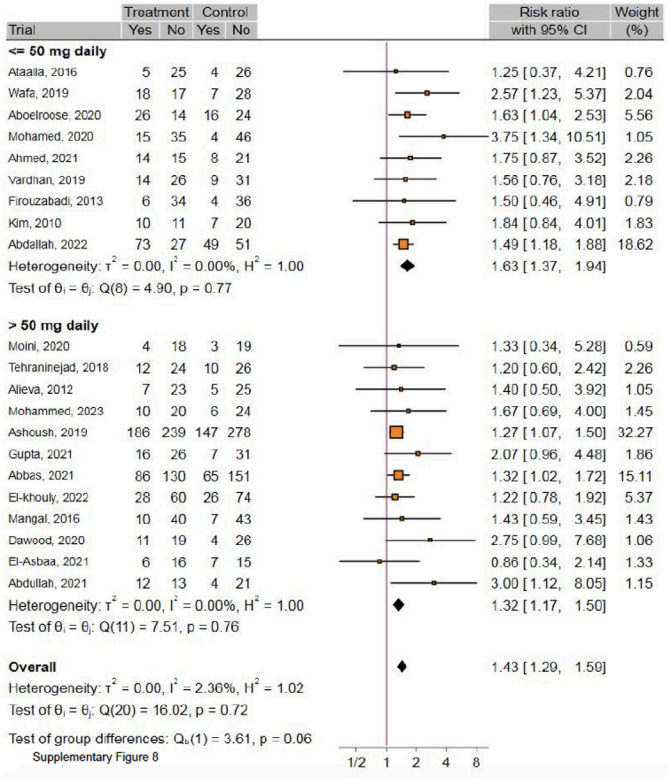


Supplement Figure 6. Leave-one-out plot showing the effect of each study on pooled estimate in subset of trials that evaluated the risk of clinical pregnancy in women who received treatment versus control

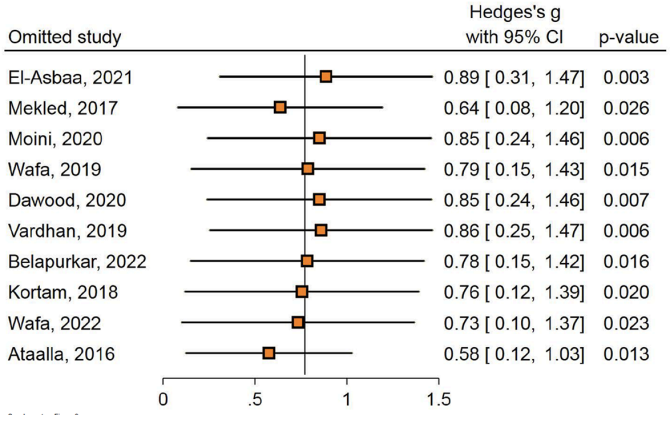
CI: Confidence interval



Supplement Figure 7. Forest plot showing individual and combined effect size estimates and 95% confidence intervals (CIs) in trials that evaluated the risk of clinical pregnancy in women who received intervention versus control regarding population type

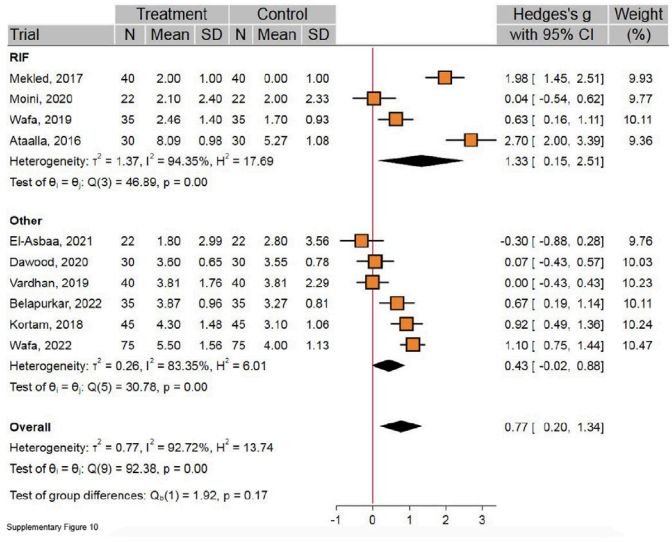


Supplement Figure 8. Forest plot showing individual and combined effect size estimates and 95% confidence intervals (CIs) in trials that evaluated the risk of clinical pregnancy in women who received intervention versus control regarding dose of sildenafil citrate



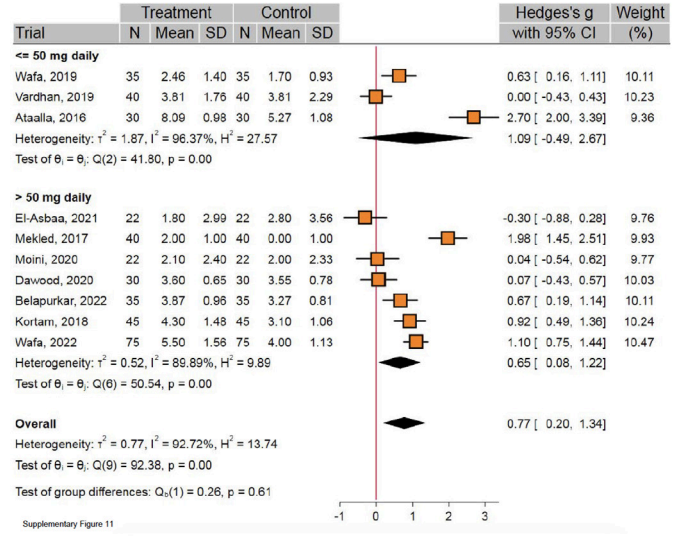
Supplement Figure 9. Leave-one-out plot showing the effect of each study on pooled estimate in subset of trials that evaluated the mean of endometrial thickness in women who received treatment versus control

CI: Confidence interval



Supplement Figure 10. Forest plot showing individual and combined effect size estimates and 95% confidence intervals (CIs) in trials that evaluated the mean of endometrial thickness in women who received intervention versus control regarding population type

SD: Standard deviation



Supplement Figure 11. Forest plot showing individual and combined effect size estimates and 95% confidence intervals (CIs) in trials that evaluated the mean of endometrial thickness in women who received intervention versus control regarding dose of sildenafil citrate

SD: Standard deviation

Review / Derleme

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Individual effects of GSTM1 and GSTT1 polymorphisms on the risk of polycystic ovarian syndrome: A systematic review and meta-analysis

GSTM1 ve GSTT1 polimorfizmlerinin polikistik over sendromu riski üzerindeki etkileri: Sistematik bir inceleme ve meta-analiz

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Abstract

This study aimed to understand the relationship between two specific genetic variations (GSTT1 and GSTM1 polymorphisms) and the risk of developing polycystic ovarian syndrome (PCOS). PCOS is a common endocrinologic disorder that affects women. Oxidative stress may play a significant role in the development of PCOS. Certain enzymes, such as glutathione S-transferases, help protect cells against oxidative stress. However, previous research on the correlation between these specific genetic variations and PCOS risk has produced inconsistent findings. To address this, a meta-analysis was conducted to examine the potential impact of these genetic variations on PCOS. We conducted a thorough search of the Embase, PubMed, Scopus, Web of Science, and Google Scholar databases to find studies that met our criteria. We used fixed-effects or random-effects models to determine the pooled odds ratios (ORs) and 95% confidence intervals (CIs) of the GSTT1 and GSTM1 polymorphisms related to PCOS. We also performed subgroup analyses based on ethnicity, mean age of participants, and PCOS diagnostic protocols. After screening, we found five studies with 1.607 participants (872 in the PCOS group and 735 in the control group) to be suitable for our meta-analysis. Our analysis showed that GSTM1 and GSTT1 null genotypes were not linked to an increased risk of PCOS (OR: 0.925, 95% CI: 0.755-1.134; OR: 1.175, 95% CI: 0.614-2.247 respectively). Additionally, both Begg's and Egger's tests revealed no publishing bias. This meta-analysis confirmed that there is no association between GSTM1 and GSTT1 polymorphisms and an increased risk of PCOS. However, further studies are required to validate this conclusion.

Keywords: GSTM1, GSTT1, polymorphism, polycystic ovarian syndrome

Öz

Bu çalışmada, iki spesifik genetik varyasyon (GSTT1 ve GSTM1 polimorfizmleri) ile polikistik over sendromu (PKOS) gelişme riski arasındaki ilişkiyi anlamayı amaçladık. PKOS kadınları etkileyen yaygın bir endokrinolojik hastalıktır. Oksidatif stres PKOS gelişiminde önemli bir rol oynayabilir. Glutatyon S-transferazlar gibi belirli enzimler hücrelerin oksidatif strese karşı korunmasına yardımcı olur. Bununla birlikte, bu spesifik genetik varyasyonlar ile PKOS riski arasındaki korelasyona ilişkin önceki araştırmalar tutarsız bulgular ortaya çıkarmıştır. Bu konuyu ele almak için, bu genetik varyasyonların PKOS üzerindeki potansiyel etkisini incelemek üzere bir meta-analiz yapıldı. Kriterlerimize uyan çalışmaları bulmak için Embase, PubMed, Scopus, Web of Science ve Google Scholar veritabanlarında kapsamlı bir araştırma yaptık. PCOS ile ilgili GSTT1 ve GSTM1 polimorfizmlerinin havuzlanmış olasılık oranlarını (OR'ler) ve %95 güven aralıklarını (GA'lar) belirlemek için sabit etkiler veya rastgele etkiler modelleri kullandık. Ayrıca etnik kökene, katılımcıların ortalama yaşına ve PKOS teşhis protokollerine göre alt grup analizleri de yaptık. Tarama sonrasında 1.607 katılımcıyla (PKOS grubunda 872 ve kontrol grubunda 735) beş çalışmanın meta-analizimize uygun olduğunu gördük. Analizimiz, GSTM1 ve GSTT1 null genotiplerinin artan PKOS riskiyle bağlantılı olmadığını gösterdi (sırasıyla OR: 0,925, %95 GA: 0,755-1,134; OR: 1,175, %95 GA: 0,614-2,247). Ek olarak hem Begg's hem de Egger's testleri herhangi bir yayın yanlılığı ortaya çıkarmadı. Bu meta-analiz, GSTM1 ve GSTT1 polimorfizmleri ile artan PKOS riski arasında bir ilişki olmadığını doğruladı. Ancak bu sonucu doğrulamak için daha ileri çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: GSTM1, GSTT1, polimorfizm, polikistik over sendromu

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Introduction

Women of childbearing age with polycystic ovarian syndrome (PCOS) experience various clinical symptoms because of this complex endocrine disorder[1]. The condition is characterized by hyperandrogenism, ovarian dysfunction, and enlarged ovaries with multiple 2-9-mm- sized follicles[2,3]. According to the World Health Organization, 3.4% of women worldwide have PCOS, which amounts to over 116 million women[4].

The cause of PCOS is not fully understood, but researchers believe it results from a combination of genetic and environmental factors, including obesity, lifestyle, ovarian dysfunction, hypothalamic-pituitary abnormalities, and oxidative stress[5]. PCOS is widely recognized as the primary contributor to menstrual irregularity, resulting in subfertility[6]. Numerous studies have provided evidence regarding the influence of genetic factors on women's embryonic development and subfertility[7,8]. Some genetic conditions may prevent fertility or improve the effectiveness of treatments for subfertility; therefore, research in this field remains highly intriguing[7,9].

Oxidative stress, acknowledged to have a crucial impact on the pathophysiology of PCOS, is an inequality between oxidants and antioxidants within cells[10]. This condition causes the accumulation of free radicals such as reactive oxygen species (ROS) and peroxides in the cells and DNA damage[11]. Intricate enzymatic and non-enzymatic antioxidant systems regulate ROS production within cells. Among these systems, the enzyme GST plays an essential role in the detoxification of ROS and in defending against oxidative stress and tissue damage[12].

In the human species, GST enzymes comprise eight distinct classes, classified according to their amino acid sequences. These classes include GSTA, GSTM, GSTP, GSTT, GSTK, GSTZ, GSTO, and GSTσ[13]. Epidemiological research has demonstrated that deletion polymorphisms of the genes *GSTM1* and *GSTT1* are prevalent within human populations and have been broadly studied[14].

At present, a limited quantity of research exists investigating the correlation between genetic polymorphisms of GSTT1 and GSTM1 and PCOS. Despite the importance of *GSTM1* and *GSTT1* gene variations and some discrepancies found in previous research, this study seeks to perform a meta-analysis of the data available to determine the influence of these genetic polymorphisms on PCOS.

Materials and Methods

Design and Search Strategy

The investigation followed the PRISMA guidelines for systematic reviews and meta-analyses[15]. A thorough search was conducted on various databases, including Embase, PubMed, Scopus, and Web of Science, to identify eligible studies that explored the possible association between GSTM1 present/null and GSTT1 present/null with PCOS risk. The studies were published up to September 2023. In addition, relevant studies were manually searched on 30 pages of Google Scholar, and the references of the selected articles were carefully checked to identify any further relevant publications. Language restrictions were not applied during the search, and the following keywords were used: ("Glutathione S-transferase" or "GST", "GSTM1" or "GSTT1") and ("Polymorphism*") and ("Polycystic ovarian syndrome" or "PCOS").

Inclusion and Exclusion Criteria

This study focused on research that met specific criteria, including the use of case-control or cohort study designs, the provision of genotype data or odds ratio (OR) with a 95% confidence interval (CI), and the examination of the correlation between GSTM1 and/or GSTT1 polymorphisms and the risk of PCOS. Studies that contained overlapping data, case reports, editorials, reviews, letters, and meta-analyses or did not provide adequate data were not included.

Data Extraction and Quality Assessment

Two authors independently extracted and reviewed the data from each study included in the meta-analysis. The extracted data are presented in Tables 1 and 2. The quality of each case-control study was assessed using the Newcastle-Ottawa Quality Assessment Scale (NOS)[16], as shown in Table 2, with a maximum total score of nine. Articles with a NOS score of seven or higher were considered high quality, whereas those with scores ranging from 5 to 7 were moderate quality.

Table 1. Basic information about the included studies

First author	Year	Country	Ethnicity	Genotyping method	Cases mean age	Controls mean age	PCOS diagnosis protocol
Babu et al.[17]	2004	India	Asian	PCR	26.5	26.4	Sonography and ultrasound scan
Savić-Radojević et al.[18]	2018	Serbia	European	PCR	16.3	16.7	2003 Rotterdam criteria
Chung et al.[19]	2020	Korea	Asian	PCR	-	-	2003 Rotterdam criteria
Azevedo et al.[20]	2020	Brazil	South-American	PCR	26.0	31.0	2003 Rotterdam criteria
Alves et al.[21]	2020	Portugal	European	PCR	33.0	31.0	Amsterdam ESHRE/ARSM-Sponsored 3rd PCOS Consensus

PCR: Polymerase chain reaction, PCOS: Polycystic ovarian syndrome

Table 2. The scores were related to the quality assessment of the eligible studies and the details regarding the included patient and control groups

First author (year)	NOS score	Case number	Control number	Case GSTM1		Control GSTT1	
				Present	Null	Present	Null
Babu et al.[17]	7	180	72	151	29	60	12
Savić-Radojević et al.[18]	8	35	17	15	19	10	7
Chung et al.[19]	7	478	376	252	226	184	193
Azevedo et al. [20]	8	110	109	129	90	60	50
Alves et al.[21]	8	69	161	34	35	89	72

NOS: Newcastl-Ottawa quality assessment scale

Statistical Analysis

In this study, we used CMA 3.0 software developed by Biostat, USA to analyze our data. Our goal was to investigate the correlation between GSTM1 and GSTT1 polymorphisms and PCOS. To determine statistical significance, we calculated ORs and 95% CIs. A p-value 0.05 indicated a significant result. We also evaluated the presence of interstudy heterogeneity using I2 and p-values. A value of I^2>50% or Q statistic test indicated heterogeneity, whereas a p-value greater than 0.10 for the Q statistic indicated its absence. We used either a random-effects model or a fixed-effects model depending on the presence or absence of heterogeneity In addition, we conducted a meta-regression analysis to explore the effects of participants' age, the method of PCOS diagnosis, and ethnicity. To ensure the reliability of our findings, we conducted a sensitivity analysis. Finally, we assessed potential publication bias using Egger's regression analysis and Begg's funnel plot.

Results

Study Characteristics

Tables 1 and 2 provide a clear summary of the clinical and demographic characteristics of the patients included in this study. The investigation consisted of five case-control studies, with 872 cases and 735 controls. The primary focus of this study was to explore the relationship between GSTM1 and GSTT1 null deletion polymorphisms and the risk of PCOS[17-21]. Table 1 shows that two studies were conducted in Asia, two in Europe, and one in South America. Figure 1 depicts a flow diagram that follows the PRISMA guidance for the literature review[15].

Quantitative Synthesis

Based on statistical analysis, it was found that the absence of the *GSTM1* gene was not significantly linked with PCOS (OR: 0.925, 95% CI: 0.755-1.134) as shown in Figure 2. Similarly, the absence of GSTT1 did not significantly increase the risk of PCOS (OR: 1.175, 95% CI: 0.614-2.247) as shown in Figure 2.

Heterogeneity Test

Table 3 shows that there was variation in the GSTT1 variate between studies, indicating heterogeneity. To investigate this further, subgroup analyses were conducted to examine the

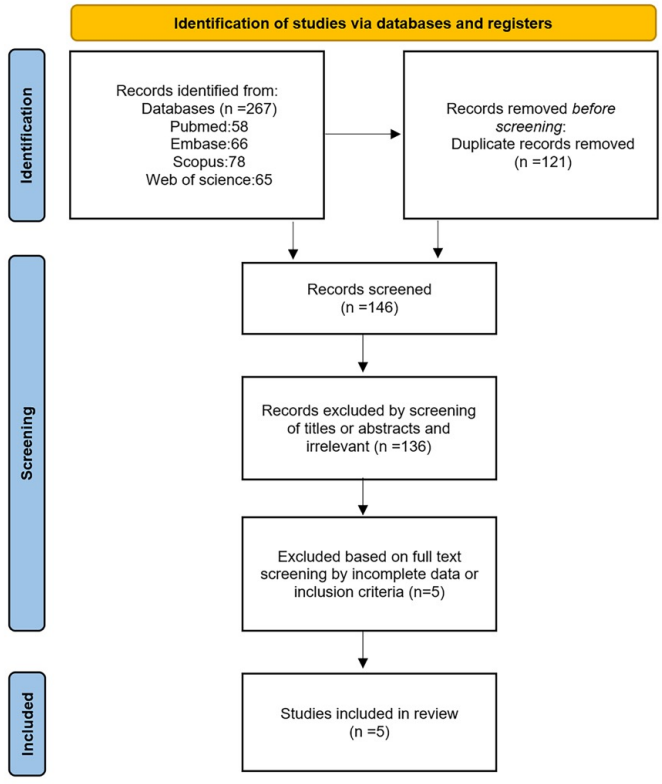

Figure 1. Flowchart of the search strategy

impact of different ethnic groups and PCOS diagnostic protocols on the results of the meta-analysis. However, no significant differences were found among the investigated ethnic groups (p=0.625) and PCOS diagnostic protocols (p=0.458), indicating that they were not the cause of the heterogeneity. Additionally, inter-study heterogeneity in relation to GSTM1 was found to be statistically insignificant (Table 3). Furthermore, the effect of the mean age of study participants on the meta-analysis results for GST1 was investigated using a meta-regression method, and the results showed that no changes were not statistically significant (p=0.1928).

Sensitivity Analysis

We conducted a sensitivity analysis to evaluate how each study affected the results of our meta-analysis. We excluded

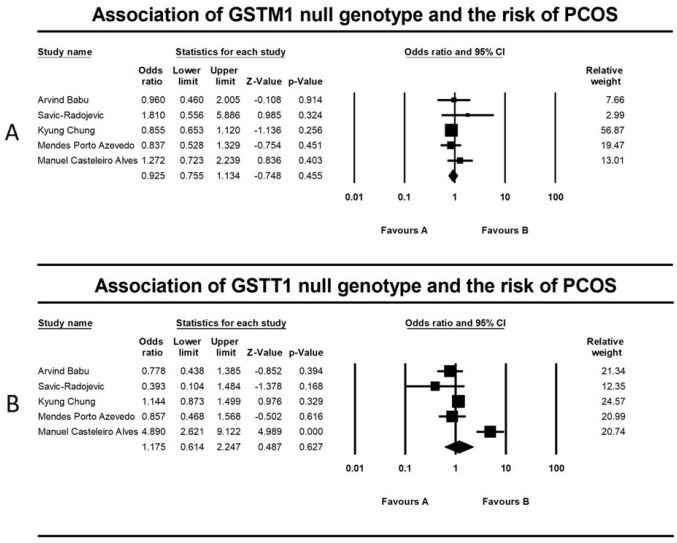

Figure 2. Forest plots of the pooled odds ratios indicating the risk of PCOS related to the null genotypes of GSTM1 (A) and GSTT1 (B)

PCOS: Polycystic ovarian syndrome, CI: Confidence interval

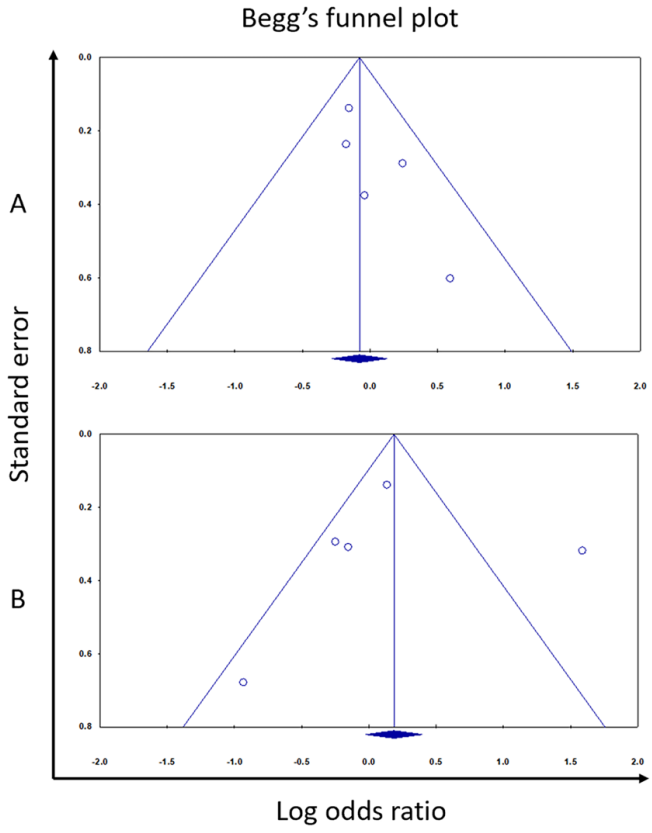

Figure 3. Begg's funnel plots for publication bias test of the studies that investigated the polymorphisms of GSTM1 (A) and GSTT1 (B)

Table 3. Heterogeneity analysis of the investigated studies

Variables	I2	Q-test's p-value	p-value of Begg's test	p-value of Egger's test
GSTM1	0.000	0.561	0.220	0.123
GSTT1	84.42%	<0.001	0.806	0.944

one study at a time and found that the combined odd ratios remained consistent. This highlights the statistical reliability of our findings.

Publication Bias Analysis

We created funnel plots (see Figure 3) to check for publication bias. Additional investigation using Egger's regression and Begg's rank correlation tests revealed no significant publication bias for GSTM1 (p=0.220; p=0.123) and GSTT1 (p=0.806; p=0.944). However, because the funnel plot for GSTT1 appears uneven, we used the non-parametric "trim and fill" test to correct for potential publication bias, but the results did not change (data not shown).

Discussion

The enzyme GST is a member of the supergene family of phase II metabolic enzymes, holds a pivotal position in detoxification processes, and plays a fundamental role in decreasing oxidative stress[22]. The most important isozymes are GSTM1 and GSTT1, which exhibit a high degree of polymorphism[23]. Several studies have presented evidence indicating that null genotypes of GSTM1 and GSTT1 may be associated with an increased risk of female reproductive system diseases as well as PCOS[24-27]. Nevertheless, the outcomes of the studies related to PCOS have not been systematically summarized and analyzed, and the overall effect remains uncertain.

This study did not reveal any statistically significant correlation between the null genotypes of GSTM1 and GSTT1 and the occurrence of PCOS. The findings of our investigation agree with those of most prior studies. Babu et al.[17], Savić-Radojević et al.[18], Chung et al.[19], and Azevedo et al.[20] are similar to the results of our study. We could not find an association between the polymorphisms of GSTM1 and GSTT1 and increased risk of PCOS. Contrary to the present study's findings, Alves et al.[21] reported a significant correlation between the null genotype of GSTT1 and susceptibility to PCOS. The most probable and reasonable explanation for the incongruous outcomes of Alves's investigation was its limited sample size, which could have led to diminished statistical potency. In another study conducted by Başkıran et al.[27], the serum level of GST was investigated, and their findings, as opposed to our study, indicated a significant reduction in the serum level of GST among patients diagnosed with PCOS compared with healthy controls. The observed dissimilarity may be attributed to the lack of examination of different GST isozymes in Başkıran's study[27], and the reduction in GST expression may be ascribed to the decline

in inoenzymes other than GSTM1 and GSTT1. The significant correlation observed between *GSP01* gene polymorphism and susceptibility to PCOS in the study by Miraghaee et al.[28] proves this claim.

After considering the overall heterogeneity, we proceeded to conduct a subgroup analysis. The results of the subgroup analysis on GSTT1 single null genotype polymorphism indicated that none of the investigated populations and PCOS diagnostic protocols did not significantly change the overall results. Accordingly, the potential source of heterogeneity is associated with sampling errors or variables other than ethnicity, diagnostic protocols, and age. However, to comprehensively scrutinize heterogeneity, further investigations should be conducted across diverse ethnic groups in the future.

This study presents several advantages. The present meta-analysis investigates the impact of null genotypes of GSTM1 and GSTT1 on the risk of PCOS for the first time. Notably, the studies incorporated in our meta-analysis were meticulously chosen based on stringent inclusion and exclusion criteria. As a result, by using high-quality articles, the credibility of our research findings was enhanced. There are some limitations that must be acknowledged, such as the limited number of primary original studies that meet the criteria for review. This poses a significant limitation in assessing interactions between genes and the environment in the progression of PCOS.

Conclusion

This meta-analysis showed that null genotypes of GSTM1 and GSTT1 may not increase PCOS risk. However, further investigations are recommended to confirm these findings.

Acknowledgments

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Ethics

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: M.H.M., S.F., Design: M.H.M., S.F., Data Collection or Processing: R.H.M., N.A., Analysis or Interpretation: M.H.M., S.F., Literature Search: M.H.M., Writing: M.H.M., S.F.

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Letter to the Editor / Editöre Mektup

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Live donor uterine transplant with vascular reconstruction: Advancing reproductive medicine

Damar rekonstrüksiyonuyla canlı donörden rahim nakli: İlerleyen üreme tıbbı

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Keywords: Uterine transplant, infertility, live donor uterine transplant
Anahtar Kelimeler: Rahim nakli, kısırlık, canlı donörden rahim nakli

Dear Editor,

Uterine transplantation (UTx) has emerged as a solitary intervention for women suffering from absolute uterine factor infertility; it effectively restores both reproductive anatomy and functionality[1]. This surgery completely transplants the uterus, cervix, surrounding ligaments, blood vessels, and a vaginal cuff, offering a substitute for surrogacy and adoption to achieve motherhood[2]. With a record of 90 cases and 49 successful deliveries, the practicability of UTx is unquestionable as it shifts from being a research concept to a fully-fledged clinical practice[1].

Recently, a groundbreaking UTx procedure was performed in the United Kingdom, featuring the use of a living donor. The recipient, a 34-year-old woman diagnosed with Type I Mayer-Rokitansky-Küster-Hauser syndrome, had no past medical and psychological records and had not undergone any prior surgical procedures. The donor, her 40-year-old sister, also had no notable medical condition and had given birth through two normal vaginal deliveries at full term. This rare case brings to light several pioneering aspects. First, it signifies the first case of vascular reconstruction within UTx. The complexity arose from the atypical vasculature, where two right-sided uterine arteries were encased into a common stem and anastomosed to the recipient's external iliac artery. Only two prior UTx cases ventured into vascular reconstruction.

Furthermore, this case introduces an innovative approach to venous drainage using the recipient's inferior epigastric vein. Traditionally, UTx procedures relied on uterine veins or utero-ovarian veins for venous drainage, which required lengthy segments of the uterine vein.

In contrast, this case sought shorter uterine vein lengths, prioritizing drainage from the utero-ovarian vessels. This unforeseen adaptation provides another option for venous drainage when conventional venous anastomoses are impossible. This case represents the first use of alemtuzumab as an induction immunosuppressive agent in UTx[3].

This milestone, marking the inaugural womb transplant, has been hailed as a fertility landmark, offering hope to numerous infertile women and ushering in a new era with the promise of enabling childbirth for many every year.

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Authorship Contributions

Surgical and Medical Practices: F.A., A.W., S.T., A.T., Concept: F.A., A.W., S.T., A.T., Design: F.A., A.W., S.T., A.T., Data Collection or Processing: F.A., A.W., S.T., A.T., Analysis or Interpretation: F.A., A.W., S.T., A.T., Literature Search: F.A., A.W., S.T., A.T., Writing: F.A., A.W., S.T., A.T.

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