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Up to date on oral misoprostol for induction of labour: an expert opinion on its use in Italian clinical practice

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ABSTRACT

Labour induction (IOL) is a standard practice in obstetrics, with the aim to facilitate the initiation of labour for obstetrical, maternal, or foetal indications. Among pharmacologic agents, oral misoprostol has emerged as a leading choice due to its efficacy, safety, compliance, and good outcomes compared to other methods. Recent meta-analyses have demonstrated the benefits of low-dose oral misoprostol (≤ 50 mcg) in reducing caesarean delivery rates while maintaining a favourable safety profile. Clear indications about monitoring and a systematic guideline about the use of this drug do not exist yet. This article presents a comprehensive overview of the clinical evidence, expert recommendations, and practical applications of oral misoprostol for IOL, with a particular focus on dosing protocols, monitoring strategies, combination with mechanical methods and women's opinion about the method.

INTRODUCTION

Induction of labour (IOL) is the process for artificially stimulating uterine contractions in pregnant women to start the labour and to achieve a vaginal delivery ideally within 24-48 hours, even if a definite cut-off of time to delivery does not exist [1, 2]. In the recent decades, induction rates increased expo-

nentially, due to the advanced maternal age and to the number of high-risk pregnancies. According with data from CeDAP (Certificato di Assistenza al Parto) 2023, IOL has nowadays a prevalence of 33.7% in Italy [3].
IOL is indicated for many obstetric, maternal, and foetal indications when the risk of continuing the pregnancy outweighs the risks of induction for the mother and/or for the foetus. Once after a shared decision-making process the clinician opts for labour induction, IOL typically includes cervical ripening, if needed, followed by the stimulation of uterine contractions and the subsequent management of latent and active phases of labour. When cervical ripening is indicated, mechanical and/or pharmacological (prostaglandins) interventions can be used [1].
Misoprostol is a synthetic analogue of prostaglandin (PG) E1 that is actually the only drug available for IOL that acts by two different mechanisms: firstly, it promotes cervical ripening by softening the cervix through the degradation of collagen in the connective tissue stroma; secondly it allows myometrial contractions through binding to PG receptors at the uterine smooth muscle cells [4, 5].
Many trials have evaluated the efficacy and safety of misoprostol for IOL. A systematic review by Alfirevic *et al*. of 76 randomized controlled trials (14,412 women) demonstrated that oral misoprostol is non-inferior to other methods in terms of efficacy, with a lower rate of caesarean sections compared to vaginal dinoprostone (RR 0.88, 95%CI 0.78-0.99) [6]. A recent meta-analysis by Kerr *et al*. also supported the use of low-dose oral misoprostol (50 mcg or less), showing that it resulted in fewer caesarean deliveries and increased vaginal births compared to vaginal dinoprostone, oxytocin, and the transcervical Foley catheter. Furthermore, oral misoprostol was associated with a lower incidence of hyperstimulation with foetal heart rate changes compared to vaginal misoprostol. Despite vaginal misoprostol leads to higher rates of vaginal delivery within 24 hours, oral misoprostol provided comparable outcomes with the additional benefit of fewer hyperstimulation-related foetal heart rate changes [7]. These findings suggest that low-dose oral misoprostol is an effective and safe choice for IOL, reducing the incidence of caesarean sections and promoting vaginal deliveries [6, 7].
In summary, Italian guidelines promoted by Ragonese Foundation report that the strength of the recommendations and the level of evidence regarding the use of misoprostol, demonstrates its efficacy and superiority compared with PGE2-based drugs [8].
As shown, the literature supporting the use of misoprostol for IOL is extensive, but these trials have been conducted using a wide variability in term of protocols.
Furthermore, the available data does not provide mandatory guideline regarding the timing of monitoring during misoprostol-induced labour. For this reason, the Italian Society of Perinatal Medicine (SIMP) has recently issued recommendations on monitoring timing during labour induction with various pharmacological methods, emphasizing that in the case of misoprostol, monitoring with cardiotocography should be performed prior to the initiation of the procedure as usual, 30-60 minutes after the first dose considering the peak of action of the active molecule. Thereafter the monitoring with cardiotocography may be based solely on clinical indications [9].
In this view the aim of our study is to provide a practice guideline about the use of oral misoprostol for induction of labour.

METHODS

A Panel of Italian Expert evaluated and investigated the rational basis and best practices for the use of oral misoprostol in pregnant women for cervical maturation and IOL in Italy, based on a wide clinical experience on that subject.
During the Expert Meeting, the following issues regarding the use of oral misoprostol were discussed:

- indications for IOL in childbirth and role of misoprostol;
- induction protocols and dosage schedule;
- cardiotocography monitoring;
- combination of misoprostol with other methods;
- role of woman's preference/experience in the choice of method for IOL.

Indications for IOL in childbirth and use of misoprostol

Expert opinion

It is essential to establish clear indications for IOL to minimize the risk of inappropriate inductions, associated complications, and failure induction rates. While reducing the induction rate may be desirable, it is important to acknowledge that, due to the change in population, an increase in the rate of

inductions is inevitable. When induction is indicated, misoprostol (alone or associated with mechanical methods) is generally the preferred method, whenever feasible. Specifically, patients who tend to benefit most from misoprostol compared to other induction methods, particularly pharmacological agents, include nulliparous women with an unfavourable Bishop score (BS) and obese patients. Some experts advise caution in using misoprostol in cases of foetal growth restriction (FGR) and, more broadly, in situations where there is a high risk of hypoxia due to chronic placental insufficiency.

The main universally accepted indications for IOL are [2, 10-13]:

- Post-term pregnancy (≥ 41 weeks);
- PROM at term (≥ 37 weeks). Generally, within 12-24 hours if the vaginal-rectal swab for group B S*treptococcus haemolyticus* is negative and within 6 hours if the swab is positive;
- Foetal growth restriction (FGR);
- Gestational diabetes (GDM)/pre-pregnancy diabetes (type I and II);
- Hypertensive disorders of pregnancy;
- Oligo/anhydramnios;
- Isoimmunization;
- Pregnancy cholestasis;
- Twin pregnancy (38 weeks Bichorionic, 36-37 weeks Monochorionic);
- Specific indications for maternal diseases (cardiac, renal, pulmonary…);
- Intrauterine foetal death.

New and discussed indications for IOL have been identified over the years:

- Obesity (especially Class II and III);
- Advanced maternal age (> 43 years)/heterologous assisted reproductive technology (ART);
- Suspected foetal macrosomia;
- Small for gestational age foetus (SGA).

The "induction rate" varies significantly across regions and should not be considered a reliable indicator of healthcare quality. In fact, a reduction in the induction rate does not inherently translate into improved maternal health outcomes within our population [3]. Rather than focusing on lowering the induction rate, it would be more beneficial to enhance clinical practices in the choice of appropriate indications and in the management of induced labour. By optimizing care, we can more effectively reduce unnecessary caesarean deliveries and improve overall maternal and neonatal outcomes. A strong antenatal counselling should be performed also in pregnancies complicated by life-limiting malformations, to obtain an effective IOL with a safe vaginal delivery [14-17].

According to the Expert Panel, oral misoprostol is the first choice among induction methods for the following reasons [6, 7, 18]:

- efficacy in terms of timing between induction start and delivery (time to delivery); number of vaginal deliveries within 24 h; caesarean delivery rate;
- safety: lower risk of uterine hyperstimulation and obstetric complications compared with dinoprostone and lower number of vaginal visits in case of PROM;
- direct action on myometrium and induction of contractile activity as well as cervical changes, resulting in less use of oxytocin during labour;
- good compliance (the oral route is preferred by women respect to the vaginal route or mechanical methods).

Current absolute contraindications to misoprostol use are few; in particular, previous uterine scars (no data are available) and contraindications to vaginal delivery, while relative contraindications are twin pregnancy, gestational age < 37 weeks, parity ≥ 4, even if in these cases, if well selected, the use of misoprostol can be considered. Oral misoprostol is not contraindicated in case of HDP and FGR, even if in most severe cases caution is recommended.

However, the main indications for misoprostol (post-term pregnancy, PROM > 37 weeks, GDM/obesity, advanced maternal age/heterologous ART, maternal diseases, cholestasis) account for about 75% of the total number of inductions. Therefore, misoprostol may be the first choice in three quarters of cases requiring IOL.

Obesity in pregnancy is an increasingly concerning issue, associated with higher rates of gestational hypertensive disorders, diabetes, foetal growth abnormalities and a greater need of induction of labour (IOL). Obese women also have higher rates of IOL failure [19, 20]. In comparison to non-obese, obese women experience a significantly longer first stage of labour, particularly a prolonged latent phase, which may increase the risk of caesarean delivery, maternal bleeding, and chorioamnionitis [21]. As the prevalence of obesity rises, achieving vaginal delivery becomes increasingly important due to the associated risks of caesarean delivery in this population. A study investigating the effect of obesity on misoprostol efficacy found that obese women, due to a larger volume of distribution, have lower bioavailability of misoprostol, poten-

tially reducing its effectiveness. This suggests that a higher dose of misoprostol may improve outcomes in obese women [22]. Additionally, a retrospective trial comparing misoprostol and dinoprostone for IOL in 564 obese patients showed that misoprostol was more effective, with higher cervical ripening rates (78.1% *vs* 66.7%; p = 0.002) and lower caesarean section rates (39.1% *vs* 51.3%; p = 0.003) compared to dinoprostone. Misoprostol was better tolerated and had similar peripartum complication and neonatal outcomes [23].

Induction protocols with misoprostol and dosage schedule

Expert opinion

- Both schedules of oral misoprostol (25 mcg every 2 hours or 50 mcg every 4 hours, up to a maximum of 200 mcg in 24 hours) [24, 25] are considered effective and feasible. In Italy, Angusta® (misoprostol 25 mcg tablet) is only authorized for induction of labour; any other use is considered off-label [24]. The choice of the regimen is made based on several factors such as the type of patient and the organization of the hospital.
- It is possible to repeat a second cycle of induction 12 hours after the last dose of the first cycle, if the first cycle did not result in the onset of labour. It is necessary not to exceed 200 mcg even on the second day of induction. According to the Scandinavian protocol [18], a third cycle is possible, but it is not currently used by the Expert Panel.
- In women with BMI > 30, the misoprostol schedule 50 mcg every 4 hours for 4 administrations is generally preferred, even if, since the daily dosage of the drug does not change, no significant differences have been demonstrated in terms of vaginal delivery rates. A dosage of 25 mcg seems to be more favourable from a pharmacokinetic perspective, ensuring a stable blood concentration (misoprostol has a peak at 12-60 minutes and a rapid concentration drop within 120 minutes) [6, 7].

The dual dosing regimen (25 mcg and 50 mcg) of misoprostol can be tailored based on clinical case and the internal protocols of the hospital. Many experts utilize both dosing schedules, as they demonstrate equivalent efficacy but allow for adjustment according to the specific needs of different patient populations.

In some clinical settings, when the 50 mcg dose of misoprostol is administered every 4 hours, a reduction to 25 mcg every 2 hours may be considered if there is evidence of significant uterine contractility along with a favourable Bishop score. This adjustment is intended to minimize the risk of tachysystole while continuing induction to optimize the results.

Regardless of dosages, induction with misoprostol should not be interrupted until the onset of labour.

Cardiotocography (CTG) monitoring during misoprostol administration

Expert opinion

- The misoprostol 25 mcg tablets SmPC does not specify the frequency of CTG monitoring [24]. According with SIMP consensus, the Experts suggest that monitoring should be carried out before starting the procedure, as usual, and again 30-60 minutes after the initial dose, considering the peak effect of the active compound. Subsequently, CTG can be performed based on clinical indications and at the onset of a regular contractile activity.
- It is crucial to stratify the risk for both mother and foetus. In case of conditions that may impair foetal tolerance to contractions, such as preeclampsia, FGR, abnormal Doppler velocimetry, or early gestational age, or factors that could increase the risk of tachysystole such as polyhydramnios, high parity (≥ 3), or twin pregnancies, an individualized CTG monitoring is warranted. In addition to CTG, careful clinical surveillance of the woman's overall well-being and uterine tone is essential throughout the induction process. If contractile activity is concerning, CTG monitoring may be initiated outside of the standard protocol to ensure the safety of both the mother and the foetus.

The requirements for proceeding to IOL include the availability of the CTG; qualified personnel to monitor the progress of the clinical picture, particularly in relation to blood loss, rupture of membranes and onset of labour; healthcare staff able to perform an emergency caesarean delivery; and a protocol for the management of tachysystole [9].

The effect of the drug on the foetal well-being is mediated by uterine contraction. During the drug administration, the contractile response of the uterus should always be manually assessed between administrations. Administration should not be "automatic" but only performed after exclusion of regular uterine activity. In case of misoprostol 25 mcg tablets administration, the amount of mi-

soprostol is guaranteed by the manufacturer and unintended overdosages are not expected.
Foetal and maternal monitoring is to be repeated based on additional clinical indications or at the onset of a regular contractile activity, also considering the risk factors that led to induction. To evaluate the contractile activity, CTG can be used if clinical monitoring is not feasible.
The use of CTG is crucial for identifying even minimal abnormalities of foetal response to uterine activity. However, clinical surveillance is often overlooked. All participants agree that clinical monitoring is equally important alongside CTG. It is insufficient to solely assess the CTG; regular clinical examinations of the woman, with detailed documentation of each evaluation or visit in the medical records, are essential for comprehensive care.
The minimum CTG monitoring time for the assessment of foetal well-being is 30 minutes after misoprostol administration. However, some variability in the duration of CTG monitoring was observed among Experts (up to 45 minutes after administration to intercept the peak concentration of misoprostol).

Combination of misoprostol with other methods
Expert opinion
The concomitant use, adopted by some Experts of the Panel, is performed by two protocols:
- mechanical method and misoprostol starting at the same time;
- mechanical method alone first and misoprostol administered after 6-12 hours, keeping the mechanical method in place.

The concomitant use of mechanical methods and misoprostol starting at the same time reduce the time to delivery compared with the sequential strategy; maternal and foetal outcome are similar to that observed with mechanical method alone. The mean rate of caesarean delivery with mechanical methods and misoprostol was 27% [26-29].
The sequential use is based on the positioning of the mechanical method for 12 to 24 hours and on the subsequent administration of oral misoprostol if BS < 6 at the time of removal of the mechanical method.
The combination of misoprostol with the mechanical method (either simultaneously or sequentially) is particularly recommended for cases with an unfavourable Bishop score and in obese patients.
A clinical trial compared the efficacy of low-dose oral misoprostol (25 mcg every 2 hours) combined with a Foley catheter to oral misoprostol alone for labour induction (IOL) at term in 200 women. The group of the combined IOL demonstrated significantly shorter intervals between induction and active delivery (10.67 *vs* 16.28 hours), induction and full dilation (11.49 *vs* 19.00 hours) and induction and delivery (16.85 *vs* 21.90 hours) compared to the misoprostol-only group. Additionally, a higher proportion of women in the combined group delivered vaginally within 24 hours. These results suggest that the combination of oral misoprostol and a mechanical method may be superior in labour induction [26].
Other studies also support the combined use of misoprostol and mechanical methods for IOL, reporting improved clinical outcomes, such as reduced induction-to-delivery time, less need for oxytocin, and fewer complications, particularly in high-risk cases [27-29].
Obese women appear to benefit most from the combined approach. Literature confirms that, while the rate of caesarean deliveries and adverse outcomes is comparable to misoprostol alone, the time to delivery is reduced with the combined method. A cohort study by Kehl *et al*. evaluated IOL in obese women (BMI > 35 kg/m^2) using a double-balloon catheter followed by oral misoprostol, if necessary, compared to misoprostol alone. The combination group had a significantly lower caesarean delivery rate (27.6% *vs* 37.5%, p = 0.0345), with the most pronounced reduction observed in nulliparous women (38.6% *vs* 56.9%, p = 0.0039). The abnormal CTG rate was also lower in the combination group (19.9% *vs* 30.4%, p = 0.0150). Multivariate analyses confirmed that the method of IOL, parity, and Bishop's score significantly influenced caesarean delivery rates. Thus, the sequential use of a double-balloon catheter and oral misoprostol is associated with more vaginal deliveries and fewer caesarean sections in obese women [30].

Role of woman's preference/experience in the choice of method for IOL
Expert opinion
To be positive and effective, the induction must be appropriate, informed, supported, respectful, and safe. Ensuring a positive birth experience for the woman is a goal of health care, and investing in patients' satisfaction leads to better clinical outcomes. Therefore, patients' involvement is an essential component of quality of care.
Improved communication and involvement of women in clinical decision-making is desirable.

A correct information about IOL to women and family members in weeks before the term (ideally around 36 weeks) is recommended. Topics of counselling should be: indication, timing, modality, expected time, course, possible complications, risk/benefit ratio, definition of success/failure of induction, alternative strategies in case of failed induction, pain control, monitoring in case of refusal of induction [12]. Once the woman is informed, a consent should be obtained, allowing sufficient time for the patient to decide.

It is crucial to optimize the choice of the induction method, considering the characteristics of the pregnancy and that when possible oral methods are preferred by women respect to vaginal/mechanical methods. An Italian cross-sectional study aimed at evaluating the impact of different modalities of IOL and delivery on levels of woman' satisfaction. This study showed that mode of delivery was associated with a higher rate of satisfaction among induced women. Considering mode of induction, the oral drug was associated with a higher level of satisfaction. An optimal control of pain and a quick induction were the characteristics most appreciated by women [31]. An appropriate choice of the method is associated with higher success rates of vaginal delivery, and in turn the outcome of delivery affects woman's satisfaction. The use of misoprostol can have a positive impact on both time and pain, as it significantly reduces the time to delivery and consequently the duration of pain (the need for analgesia is lower because there is less time for psychological and physical suffering and because the oxytocin use is decreased).

COMMENTS AND CONCLUSIONS

In conclusion, oral misoprostol has emerged as the first-line agent for labour induction, thanks to its proven efficacy, favourable safety profile, and patient preference. In fact, based on the current literature and considering its contraindications, misoprostol should be considered the first choice in majority of the cases where induction is indicated. Ongoing research into dosages and combined strategies, holds great promise for refining clinical practices, reducing time-to-delivery, and improving maternal and foetal outcomes in the more complex cases. In fact, tailoring of protocols is crucial to ensure "the right method for the right patient". In this way healthcare providers can enhance the overall success of the induction process.

To optimize the induction of labour, the following steps are essential:

- accurate assessment of appropriate indications for labour induction;
- selection of the appropriate induction method based on factors such as parity, Bishop score, BMI, gestational age, specific indication, and risk factors for foetal intolerance or tachysystole;
- comprehensive patient education regarding induction methods, provided before hospitalization, ideally starting from the 38th week of pregnancy;
- clear communication about the induction timeline, as many women believe that induced labour will progress rapidly;
- offering alternative methods if the initially chosen approach fails;
- assessing patient compliance with the induction plan;
- organizing the induction process based on available resources in the ward and delivery room.

COMPLIANCE WITH ETHICAL STANDARDS

Authors' contribution

All authors: Conceptualization, writing – review & editing. S.Z., A.F., L.D.: Writing – original draft.

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The impact of stress on menstrual cycle changes around examination time among medical college students: a multicentric study

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ABSTRACT

Objective. Stress was linked to many menstrual cycle (MC) abnormalities, especially during stressful periods like exams. Medical students are known to experience higher stress levels compared to other disciplines. This study primarily examined the impact of stress on MC parameters. Secondly, to verify predictors linked to stress and successful stress-coping strategies among students to mitigate stress-related MC changes.

Materials and Methods. A cross-sectional study enrolled medical college students via an online survey by Google Forms. Three data sets were collected: demographics, menstrual history, and socio-behavioural factors. 361 participants were grouped into 2: Group A, who experienced no change in MC, and Group B who did. Chi-square and odds ratio (OR) compared groups.

Results. 87.7% of participants were 22-24 years old. MC history and menstrual changes at the exam were all statistically significant across groups. In contrast, sleep and diet were not. Stressful life events and asking for medical advice were statistically significant. Reduced blood loss had highest odds for exams-related stress: OR:10.85, 95%CI 5.41 to 21.75, followed by reduced MC length: OR: 9.91, 95%CI 4.91 to 4.29. Most reliable coping strategy was asking for medical advice: OR: 2.68; 95%CI 1.68 to 4.29.

Conclusions. Exam-related stress was more evident in students with abnormal menstrual history manifested as dysmenorrhea and reduced cycle length. Most reliable stress predictors were reduced blood loss and cycle length. Identifying students most likely to suffer exam-related stress and the most effective stress-coping strategies are vital to support female students and help them achieve academic success.

INTRODUCTION

Menstruation is considered a normal physiological event in a woman's life, and it is the hallmark of normal health in terms of regularity, amount, and premenstrual symptoms preceding the menstrual periods. Normal menstrual cycle length is between 21 and 35 days, and the menstrual flow is between 2 and 7 days. Alteration of periods' normality, as well as premenstrual physical and psychological

changes, possess a significant impact on a woman's life and her productivity at levels of academic achievements, social life, and work [1].

Menstrual cycle (CM) abnormalities can be attributed to a wide variety of influencers, including stress and psychological impacts as academic, emotional, and physical intensities [2]. There are also socio-behavioural changes, such as dietary and sleep changes, caffeine, smoking, and drugs, which have a bidirectional impact on MC function. Additionally, the medical diseases and metabolic disorders that affect MC include polycystic ovarian syndrome (PCOS) as the most important moderator [1-3] and body weight changes with its detrimental effects on MC [3, 4]. A number of researchers studied the MC changes at the time of an exam to clarify the effects of the exam as a stressful event on women's performance. Some studies discussed that dysmenorrhea, lengthened cycles, were more frequently reported; others discussed oligomenorrhea as the most frequently reported symptom [4, 5]. An earlier study from Iraq reported that oligomenorrhea and menorrhagia were the most frequent MC abnormalities among medical college students [6]. Changes in food habits can be integrated with menstrual abnormalities, as highlighted by many studies that evaluated the impact of changing diet at stressful times, such as exams on the MC changes, and how lack of physical activity can exacerbate those changes [7]. Others looked into food types, particularly junk food consumption, and how they may be associated with MC rather than dietary habits [8].

Sleep health is a crucial variable for reproductive health [9]. Additionally, it should be optimized at the time of the exam to achieve the best academic results. According to the recommendations, sleeping hours should be between 7 and 9 hours per night [10]. However, deviation-reduced sleeping hours and poor sleep quality have been associated with poor performance at exams, depression, and obesity, with their detrimental effects on MC regularity and health [11]. Stressful life events experienced by the students lead to increasing levels of procrastination, with lower problem-solving abilities and an increase in negative stable attributions [12]. Support in terms of familial, social, and academic support can have a major role in modifying stress and alleviating its adverse effects on academic achievements, added to diverse coping strategies to reduce exam-related stress [13]. Despite extensive work regarding MC changes in stress, little is known about targeting specific MC changes; understanding these changes and how they adversely impact student reproductive health and overall resilience is crucial to developing successful stress-coping strategies.

This study primarily aimed to describe the impact of exam-related-stress on the menstrual cycle and various modifiable social-behavioural parameters associated with those changes. The secondary aim is to appraise successful stress predictors and coping strategies to mitigate their impact and enhance academic performance and success among Iraqi female medical students.

PATIENTS AND METHODS

Study design and setting

A cross-sectional multicentric study was conducted in major medical colleges in our country with an overall number of students of more than 2,000 to recruit female students through an online questionnaire for three months, from June to September 2024. Medical colleges have the highest academic level: they involve six grades. The first three years are basic sciences; the other three are clinical sciences, including hospital training. The study protocol was approved by the University Ethics Committee/College of Medicine (IRB Approval 2024-070, on May 27, 2024).

Study sampling and participants

The study employed stratified random sampling to recruit all female medical students in the last three years and newly graduated who completed the survey in full and agreed to participate. To avoid incomplete or duplicated responses, the questions were assigned as required so that submission was only accepted after completing all questions. The initial responses had 390 participants.

An exclusion was made for students beyond the required academic years and those who suffered chronic medical conditions and ongoing drug therapy. The final number of participants included in the analysis was 361.

Sample size calculation

The online Rao soft sample size calculator [14]. The sample size was calculated based on a response rate of 50%, a confidence interval of 98%, and a margin of error of 5%, with a total population of about 2,000. The sample size required was 350, and the final responses included were 361.

Recruitment method

Participants were recruited through an online questionnaire conducted via Google Forms distributed via social media platforms (WhatsApp and Telegram Applications) over three months from June to September 2024.

The questionnaire targeted current and recent graduates who studied obstetrics and gynaecology and cooperated with their teachers. Written informed consent was obtained from all participants following the Helsinki Declaration. It was emphasized that participation was entirely voluntary, and the anonymity and confidentiality of the data were assured, with the right to withdraw at any stage retained.

Data collection

The study tool (questionnaire) was developed after an extensive literature review and was tested by a pilot study from experts in the field before launching. According to their input, technical issues were addressed, and the original questionnaire was revised and distributed online in both English and Arabic for improved clarity and accessibility. The questionnaire consisted of three main sections, with several questions included in each section.

The first section involved the demographic variables studied, which are the students' age, age at menarche, academic year, marital status, and body mass index (BMI). Any history of chronic diseases and chronic drug use.

The second section included questions on menstrual cycle abnormalities, which include any history of menstrual abnormality, history of polycystic ovarian syndrome, amenorrhea (cessation of periods for 3 months), abnormalities around time of exam concentrating on the length of the cycle (normal, longer or shorter than before exam), amount of blood loss (normal, more than or less than previous periods) and dysmenorrhea (as no pain, mild or severe pain) around time of exam (around time of exam indicating 2 to 3 months before and after).

In our survey, we used MC definitions adopted by the International Federation of Obstetrics and Gynecologists (2018) to keep MC parameters consistent across all participants [1].

The third section involves socio-behavioural variables, which are dietary habits changes (no change, reduced or increased food intake), sleep pattern (no change, reduced sleep, poor sleep, and no sleep), stressful life events other than exam present or no, the medical branch of the exam (surgery, medicine, paediatrics, obstetrics and gynaecology, basic sciences and no change), asking for medical advice or not and taking medications or not. **Figure 1** illustrates the study sampling.

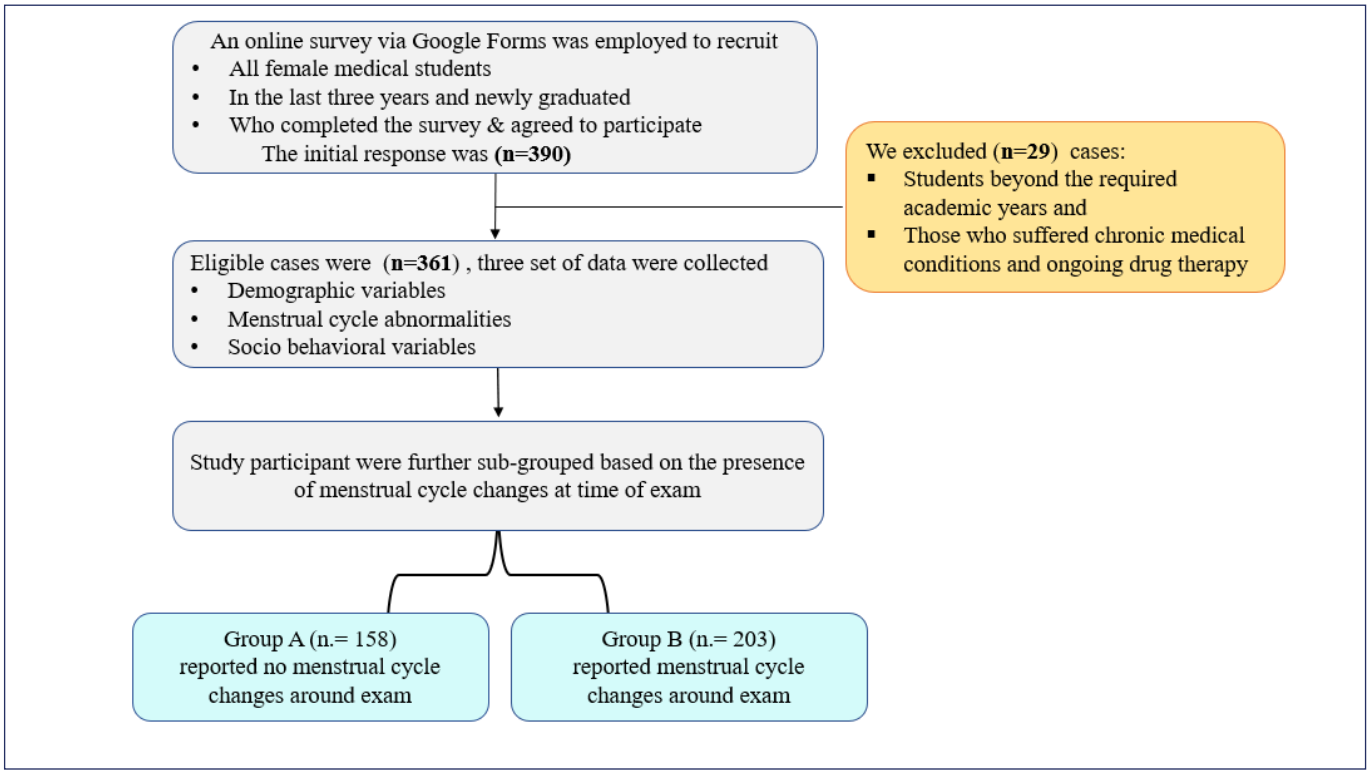

Figure 1. *Study flow chart.*

Statistical analysis

Descriptive statistics (numbers and percentages) were used to express the demographic criteria of the study population. The chi-square test detected the difference in categorial factors in study parameters. Logistic regression analysis was conducted to calculate the Odds ratio (OR) and respective 95% confidence intervals (CI) for various parameters and verify their impact on MC changes. The statistical analysis was conducted via Microsoft Excel 2016, Statistical Packages of Social Sciences-SPSS (2019), and MedCalc® Statistical Software version 22.016. The significance level was set at a P-value < 0.05 for all tests.

RESULTS

The demographic variables of the participants (n = 361) are demonstrated in **Table 1**, which revealed that 87.7% of participants were 22-24 years old; age at menarche was 12 and 13 years in 60.1%. The participants were mainly from the 6th grade (55.1%) and the 5th year (23.3%). More than 97% were unmarried. Regarding BMI, normal BMI constituted the majority (60.9%), overweight 33.8%, and the least were underweight (5.3%). **Figure 2** shows the distribution of participants based on the universities.

The study participants were sub-grouped based on the menstrual cycle (MC) changes around the exam: Group A (n = 158) reported no MC changes, while Group B (n = 203) experienced MC changes with a prevalence of 56.23% of MC changes among the whole study participants.

In **Table 2** the factors associated with MC abnormalities among the two groups were compared. A prior history of menstrual cycle abnormalities (72.41%), a history of PCOS diagnosis (21.18%), and a history of amenorrhea (14.77%) were significantly higher among Group B with P-value of 0.0005, 0.03, and 0.008, respectively.

The frequently reported MC changes around the exam time among Group B were sub-categorized as follows: normal MC length was reported at 38.42%, compared to 29.06% *vs* 32.51% of cases who reported longer and shorter MC; p = 0.0001, respectively. The amount of blood loss was normal among 37.93%, p = 0.0001 of the participants, while 34.98% *vs* 27.92% of the participants suffered increased and reduced blood loss than normal MC, p = 0.005, respectively.

Table 1. *The demographic criteria of the participants.*

Variables	Categories	Numbers	Percentages
Age (years)	20	3	1%
	21	14	3.8%
	22	94	26.1%
	23	147	40.8%
	24	75	20.8%
	25	20	5.4%
	26	8	2.1%
Age at menarche (years)	9	2	0.5%
	10	70	19.5%
	11	45	12.5%
	12	128	35.4%
	13	89	24.7%
	14	20	5.4%
	15	2	0.5%
	16	5	1.5%
Academic years	4th	14	3.8%
	5th	84	23.3%
	6th	199	55.1%
	Graduated	64	17.8%
Marital status	Unmarried	351	97.2%
	Married	10	2.8%
	Divorced	0	0%
Body Mass Index (BMI); Kg/m^2	Underweight < 18.5	19	5.3%
	Normal weight: BMI of 18.5 to 24.9	220	60.9%
	Overweight > 24.9	122	33.8%

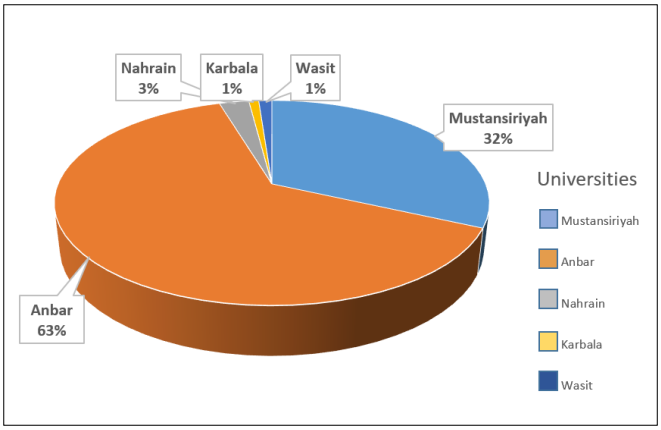

Figure 2. *Distribution of study participants based on universities' participation.*

Dysmenorrhea: a significant number of participants experienced severe and moderate dysmenorrhea, 41.87% *vs* 40.89%; p = 0.008, respectively.

Table 2. *Comparing the MC abnormalities among the two groups (n = 361) in respect to menstrual history and menstrual parameters around examination times categorized according to groups.*

Parameters	Categories	Group A n = 158 (%)	Group B n = 203 (%)	Total n = 361 (%)	P-value
Prior history of MC abnormality					
Any prior history of menstrual abnormality	Yes	135 (85.44%)	147 (72.41%)	282 (78.12%)	0.0005
	No	23 (14.56%)	56 (27.59%)	79 (21.88%)	
History of PCOS Diagnosis	Yes	23 (14.56%)	43 (21.18%)	66 (18.28%)	0.03
	No	135 (85.44%)	160 (78.82%)	295 (81.72%)	
Amenorrhea (3 months)	Yes	11 (6.96%)	30 (14.77%)	41 (11.36%)	0.008
	No	147(93.04%)	173(85.22%)	320 (88.64%)	
Prior history MC abnormality around exam time					
A. Does MC length change at the time of the exam	Normal (no change)	131 (82.91%)	78 (38.42%)	209 (57.89%)	0.0001
	Longer than normal MC	13 (8.23%)	59 (29.06%)	72 (19.94%)	
	Shorter than normal MC	14 (8.86%)	66 (32.51%)	80 (22.16%)	
B. Amount of blood loss at the time of the exam	Normal (no change)	125 (79.11%)	77 (37.93%)	202 (55.96%)	0.0001
	More than normal MC	12 (7.59%)	71 (34.98%)	83 (22.99%)	
	Less than normal MC	21 (13.29%)	55 (27.09%)	76 (21.05%)	
C. Dysmenorrhea around the time of the exam	No pain	45 (28.48%)	35 (17.24%)	80 (22.16%)	0.005
	Moderate pain	45 (28.48%)	83 (40.89%)	128 (35.46%)	
	Severe pain requires bed rest	68 (43.04%)	85 (41.87%)	153 (42.38%)	

MC: menstrual cycle changes; Group A showed no menstrual cycle changes. Group B showed menstrual cycle changes.

Examining the socio-behavioural changes among students that associate MC at the time of the exam was clarified in **Table 3**.

There was no statistical difference between the two groups concerning dietary changes, sleep disturbances, stressful life events, and the type of medical branch being examined, with a P-value of 0.45, 0.67, 0.064, and 0.058, respectively. The changes in dietary and sleep patterns were more significant among Group B. Stressful life events were reported among the two groups but were higher in Group A (70.89 *vs* 59.61%), respectively. The medical branch that was frequently reported among Group B was the surgical branch, followed by the medical branch (34.3 *vs* 32.1%); p = 0.0001, respectively.

Coping with stress strategies used by the students showed meaningful differences for seeking medical advice, reported in 77.22% *vs* 57.14%; p = 0.0007 of Group A and Group B, respectively. Using medication to reduce exam-related stress was reported in 27.22% *vs* 22.17%; p = 0.32 of Group A and B, respectively. All students (100%) sought support, primarily from family 72%, followed by friends 15%, and academic staff 12%, as illustrated in **Figure 3**. In **Table 4** a calculation of the OR and associated 95% CI was made to show predictors for the stress on MC changes. The strongest predictors were reduced blood loss, OR 10.85 (95%CI 5.41 to 21.75, p < 0.0001), followed by reduced MC length OR = 9.91 (95%CI 4.91 to 19.98; p < 0.0001). Based on the menstrual history, the best predictor was a positive history of amenorrhea with an OR of 6.65 (95%CI 1.27 to 5.62; p = 0.009). All tested parameters had a

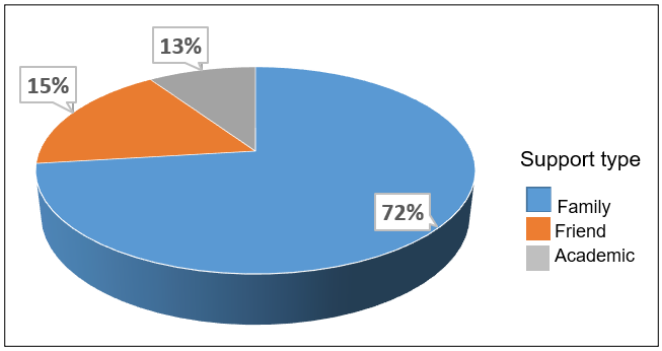

Figure 3. *Type of support received by the female student during the exam.*

Table 3. *Examining the socio-behavioural changes and coping strategies among students that associate MC at exam time.*

Socio-behavioural variables	Categories	Group A (n = 158) (%)	Group B (n =203) (%)	Total (%)	P-value
Dietary changes					
Are there any changes in dietary habits?	No change	45 (28.48%)	50 (24.63%)	95 (26.31%)	0.45
	Reduced food intake	51 (32.28%)	78 (38.42%)	129(35.73%)	
	Increased food intake	62 (39.24%)	75 (36.95%)	137(37.95%)	
Sleep changes					
Sleep disturbances	No change	14 (8.86%)	22 (10.84%)	36 (9.97%)	0.67
	Reduced sleep	77 (48.73%)	89 (43.84%)	166(45.98%)	
	Poor sleep	57 (36.06%)	74 (36.45%)	131(36.29%)	
	No sleep	10 (6.33%)	18 (8.87%)	28 (7.76%)	
Stress					
Stressful life events	Yes	112 (70.89%)	121(59.61%)	233(64.54%)	0.02
	No	46 (29.11%)	82 (40.39%)	128(35.46%)	
Which medical branch carries the highest stress at exam time	Surgery	58 (36.71%)	76 (34.44%)	134(37.12%)	0.058
	Medicine	50 (31.64%)	65 (32.02%)	115(31.86%)	
	Paediatrics	19 (12.03%)	18 (8.87%)	37 (10.25%)	
	OBG	1 (0.63%)	8 (3.94%)	9 (2.49%)	
	Basic sciences	2 (1.27%)	0 (0.00%)	2 (0.55%)	
	No change	28 (17.72%)	36 (17.73%)	64 (17.73%)	
Coping with stress					
Asking for medical advice around exams	Yes	122 (77.22%)	116 (57.14%	238(65.93%)	0.0007
	No	36 (22.78%)	87 (42.86%)	123(34.07%)	
Do you take medication	Yes	43 (27.22%)	45 (22.17%)	88 (24.38%)	0.32
	No	115 (72.78%)	158 (77.83%	273(75.62%)	

MC: menstrual cycle changes; Group A showed no menstrual cycle changes. Group B showed menstrual cycle changes.

statistically significant odd ratio except moderate dysmenorrhea with OR of 1.69 (95%CI 0.98 to 2.92). The socio-behavioural changes' best predictor was stressful life, which had an OR of 1.91 (95%CI 1.23 to 2.96; p = 0.003). None of the other examined parameters were significant. Coping with stress strategies showed that asking for medical advice was a strong predictor for stress on MC with an OR 2.68 (95%CI 1.68 to 4.29; p < 0.0001), while taking medication failed to have statistical value.

DISCUSSION

The analysis confirmed that examination-related stress was more evident in students with abnormal menstrual history and is more likely to be manifested as dysmenorrhea and reduced cycle length. None of the socio-behavioural changes (sleep and diet) were significant among the groups that showed and those that did not show MC changes. The most reliable predictors for examination-related stress were reduced menstrual blood loss and reduced cycle length. All socio-behavioural changes were non-statistically significant. The most effective stress-coping strategy was seeking medical advice.

The study highlighted that MC changes had a prevalence of 56.23% among the whole study participants. Dysmenorrhea was the most frequently reported (77.7%), followed by changes in blood loss (44.4%) with a P-value of 0.005 and 0.0001, respectively.

In **Table 5**, a summary of some of the reported MC changes across different countries was highlighted [4, 5, 15-17]. The discrepancy can be attributed to racial, genetic, socio-behavioural, and dietary factors. A prior history of MC abnormalities, amenorrhea,

Table 4. *The logistic regression was employed to calculate the OR and associated 95%CI to show predictors for stress on MC changes.*

Parameters	Categories	Wald	Odd ratio	95% CI	P-value
Menstrual cycle history					
History of MC changes: reference group (no history of changes)	Yes	11.64	2.61	1.50 to 4.53	0.0006
History of PCOS: reference group (no history of PCOS)	Yes	4.59	1.86	1.05 to 3.27	0.03
History of amenorrhea: reference group (no amenorrhea history)	Yes	2.67	6.65	1.27 to 5.62	0.009
Menstrual cycle variables					
Change in MC length: reference group (no change in MC length)	Reduced	41.06	9.91	4.91 to 19.98	< 0.0001
	Increased	43.75	9.21	4.77 to 17.77	< 0.0001
Changes in blood loss: reference group (no changes in blood loss)	Reduced	45.16	10.85	5.41 to 21.75	< 0.0001
	Increased	24.81	4.34	2.44 to 7.74	< 0.0001
Dysmenorrhea: reference group (no pain)	Moderate	3.55	1.69	0.98 to 2.92	0.058
	Severe	10.49	2.58	1.45 to 4.59	0.001
Socio-behavioural changes					
Change in diet intake: reference group (No change)	Reduced	3.19	1.63	0.95 to 2.78	0.07
	Increased	1.92	1.45	0.86 to 2.45	1.16
Sleep disturbances: reference group (No change)	Reduced	0.56	0.75	0.36 to 1.57	0.45
	Poor sleep	0.33	0.80	0.38 to 1.7	0.56
	No sleep	0.06	1.15	0.41 to 3.19	0.79
Stressful life events: reference group (positive stressful life events)	No	2.88	1.91	1.23 to 2.96	0.003
Stress coping strategies					
Asking for medical advice; Reference group (not taking)	Yes	17.24	2.68	1.68 to 4.29	< 0.0001
Taking medication; Reference group (no drug intake)	yes	0.7402	1.24	0.76 to 2.0	0.74

MC: menstrual cycle; 95%CI: 95% confidence interval.

and PCOS was significantly higher among Group B in univariant analysis. During stressful conditions, such as examinations, students with a positive history of MC changes will be more likely to experience those changes or even experience an exacerbation during stress owing to hormonal imbalance. This highlights a relationship between pre-existing MC health and the probability of reporting MC changes at stressful times. Earlier studies aligned with our results. Academic stress tends to exacerbate changes in PCOS cases, as high as 80% [3, 18].

In bivariate analysis (logistic regression model), the validity of those parameters was emphasized. Reduced blood loss during the menses, reduced MC length, and increased MC length had the highest OR in predicting MC changes among students at examination time, with OR of 10.85, 9.91, and 9.21, respectively.

The socio-behavioural variables (diet, sleep) showed a higher trend in Group B, which fails to have statistical value in univariate analysis, further confirmed by multivariant analysis with an OR of less than two and a P-value of 0.05. These results contradict earlier studies in the field that discussed an association between those variables with MC changes and exam-related stress. One study discussed higher food intake during menstruation, while another showed that fasting led to an alteration in the MC pattern [19, 20]. Higher food intake can affect the body fat composition and sex hormones by triggering a proinflammatory state, which affects the MC even in the absence of stress. In contrast, healthy food habits with high vegetable and fruit consumption have a positive influence on menstrual periods and pain owing to anti-inflammatory properties [7].

Table 5. *A summary of some of the reported MC changes across different countries was highlighted.*

Country	Sampling size	Prevalence	Most reported change	Authors, years
Iraq	361	56.23%	Dysmenorrhea	Current study
Saudi Arabia	450	48.2%	Dysmenorrhea	Alhammadi *et al.* [4], 2022
Egypt	366	33.32%	Reduced blood loss	Abdella *et al.* [15], 2016
Ethiopia	620	32.6%	Dysmenorrhea	Zeru *et al.* [16], 2021
Nepal	253	64.2%	Dysmenorrhea	Thapa *et al.* [17], 2015
India	300	35.72%	Oligomenorrhea	Kumar *et al.* [5], 2018

Changes in sleep patterns were also linked to MC irregularity in terms of poor quality of sleep or reduced sleep hours [9, 21]. Although the exact triggers are not well understood, sleep disturbances are quite common in women with MC changes. Some postulated that sleep disturbances might affect the gonadal hormones section manifested as irregular cycles [22]. The integrity of the HPA axis (hypothalamic-pituitary-adrenal) is mainly dependent on a circadian rhythm, which will be lost among those with sleep disturbance, consequently impacting gonadal hormone secretion [23].

The discrepancy in this study's result regarding diet and sleeping patterns compared to other studies could be attributed to multiple factors. One possible cause is that these variables were overshadowed by others that had more impact on the MC function [24]. Another possibility is that the impact of those two variables may be complex and multifaceted and involve an indirect pathway that can not be measured by immediate outcome [9, 19, 24]. Many studies stressed the impact of socio-behavioural parameters on reproductive and menstrual health. It is worth noting that some females may experience minimal or no changes or alterations in MC function, which indicates that there is an individual variation of response to a stressful situation [25].

Although stress had no statistical difference on univariant analysis, it showed a significant impact on multivariant analysis with an OR of 1.9 and a P-value of 0.003. Many studies increasingly recognize the impact of stress on MC wellness among medical students who were exposed to higher levels of stress (academic stress, psychological and financial stressors) compared to students in other disciplines [13, 25].

Annarahayu *et al*.'s meta-analysis declared that women experiencing stress are at higher risk (1.8% higher) to have irregular menses compared to women without stress [26].

The integrity of the HPA and hypothalamic-pituitary-gonadal axis (HPG) is vital to ensure MC cyclicity. Upon exposure to stressful life events, such as exams, the HPA will be activated, causing more stress hormones to release, such as cortisol. Consequently, the normal function of the HPG axis is inhibited, creating a functional hypothalamic dysfunction manifested as amenorrhea and reduced MC blood loss. Another proposed mechanism is the reduced luteal phase progesterone secretion in response to stress [1, 27].

The analysis showed that the surgical and medical branches were the most frequently reported branches of stress compared to other branches but without statistical value [28]. This aligns with earlier studies reporting high clinical stress rather than basic sciences [29].

Among the coping strategies to face stress, group A students were statistically higher in seeking medical advice; this was further verified in multivariant analysis with an OR 2.68 and P-value < 0.0001. This was in line with other studies which discussed that this strategy is highly valued for stress management and is used more frequently by females [30, 31]. In contrast, group B students had a trend of higher medication intake, which failed to reach statistical value. Earlier work showed that medication intake to reduce stress is not common among medical students, and it does not show a good association with stress management [32, 33].

Seeking advice from a medical professional may offer a tailored coping strategy. When paired with medication, it adds a more holistic patient approach by addressing the stress of psychological and physical symptoms [34]. It is essential to consider advanced coping strategies, such as cognitive therapy, meditation, and coaching. Increasing public awareness and education regarding them is essential for promoting their use [35].

It is noteworthy that all participants seek support from either a family member, a friend, or an acade-

mic member. This coping strategy is quite common, especially among female students who experience high-stress levels [36, 37]. A study from Iraq examined the role of mother support during exams. It declared that the mother's education level and personal history of menstrual abnormalities affected dysmenorrhea and premenstrual syndrome incidence and, consequently, academic performance and grading score [38]. Overall, fostering and promoting a supportive environment, whether at home or college, is vital to reducing exam stress and improving students' well-being and success.

We have to acknowledge the study limitation; being a cross-sectional study hinders the establishment of cause-and-effect links between study variables. Study variables were self-administered, so it is prone to recall bias and subjective interpretation. Moreover, previous studies had reported higher perceived stress levels during the luteal phase, which is a notable limitation of the current study that was not addressed. The socio-behavioural changes that this study focused on are multifaceted aspects and may not have been fully captured by the research tool. The multicentric design of the study enhances results generalization. However, the diversity of environmental factors might modulate stress and coping strategies. Thus, they may be a potential source of bias. There is a broad spectrum of coping mechanisms that might be more effective than the limited range considered in this study. The effect of other hormone that might contribute to irregular cycle was not addressed such as prolactin and cortisol [39-41]. Prolactin is one of stress related hormones, once it increased, it will suppers GnRH secretion thus subsequently reduces LH and FSH hormones. A state of anovulatory cycle will be created leading to irregular cycle or even amenorrhea. Hyperproteinemia is often linked to stress and pituitary disrobers [42, 43].

This was the first multicentric study in our country to address examination-related stress on MC changes in a large population. One of the key strengths is the holistic approach adopted; not only are menstrual variables addressed, but socio-behavioural patterns are also used to have a deeper insight into students' well-being. Furthermore, the analysis has extended to evaluate coping strategies that help students manage the stressors perceived to optimize academic performance.

To address study confounders, we focused on the 4^{th}-6^{th} year and graduated students to reduce age-related diversity and capture mature students. Additionally, an exclusion was made for students with chronic illness or those on chronic therapies. The questionnaire was designed in a way that makes the submission incomplete before all the questions are answered to limit incomplete or missing data.

The clinical impact of this study is critical as it provides the foundation for supporting female students during stressful periods and ultimately reaching their potential and academic success.

CONCLUSIONS

In conclusion, the current study investigated menstrual changes among female students in major medical colleges. It was shown that all menstrual history parameters, menstruation variables, and the presence of stress were significant. None of the socio-behavioural variables were meaningful. Reliable stress predictors were reduced menstrual blood loss and reduced cycle length.

Identification of students most likely to suffer stress and predictors for exam-related stress helps develop preventive measures and fosters supportive intervention and guidance to enhance females' academic performance. Future research is warranted to examine the long-term impact of exam-related stress on women's menstrual and psychological health and to explore more effective stress-coping strategies.

COMPLIANCE WITH ETHICAL STANDARDS

Authors' contribution

W.N., B.H.H.: Conceptualization, validation, visualization, writing – original draft. R.M.M.: Supervision, writing – review & editing. W.A.: Methodology.

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Study registration

N/A.

Disclosure of Interests

The authors declare that they have no conflict of interests.

Ethical approval

The Mustansiriyah University ethics committee approved this study (IRB Approval 2024-070, on May 27, 2024).

Informed consent
All patients gave their singed consent for publishing the images' used

Data sharing
Data are available under reasonable request to the corresponding author.

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Ablation and risk of recurrence in endometrioma: a systematic review and meta-analysis

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ABSTRACT

Objective. Ovarian endometriosis, or endometriomas, is a common manifestation and is typically treated via laparoscopic cystectomy. However, this method may compromise ovarian function. Ablation, involving thermal destruction of endometriosis cells, is emerging as an alternative. Despite some evidence suggesting less impact on ovarian function, there is a lack of robust data on the efficacy of ablation in preventing recurrence. This systematic review and meta-analysis aim to evaluate the recurrence rates of ablation compared to standard cystectomy for ovarian endometriomas.

Materials and Methods. This study followed PRISMA guidelines and was registered with PROSPERO (protocol number CRD549177). Comprehensive searches were conducted in PubMed, EMBASE, Scopus, Google Scholar, ClinicalTrials.gov, and the Cochrane Central Register up to May 2024. Inclusion criteria focused on studies involving patients with at least one ovarian endometrioma treated with ablation or cystectomy, reporting recurrence rates, and having a minimum follow-up of 12 months. The studies were assessed for quality using the Newcastle-Ottawa Scale. Data were analysed using fixed-effect or random-effect models based on heterogeneity, with statistical significance set at $p < 0.05$.

Results. The search identified 58 articles, with 16 meeting the criteria for review. 5 studies, encompassing 395 patients, were included in the final analysis. 4 studies compared ablation and cystectomy. Recurrence rates varied, with ablation ranging from 0% to 37.7% and cystectomy from 0% to 22%. Meta-analysis revealed a non-significant trend toward higher recurrence rates with cystectomy (OR 1.99, 95%CI 0.95-4.16, $p = 0.07$). The heterogeneity was low ($I2 = 0\%$, $p = 0.45$).

Conclusions. This systematic review and meta-analysis did not find a statistically significant difference in recurrence rates between ablation and cystectomy for treating ovarian endometriomas. However, there was a non-significant trend favouring ablation. Further randomized controlled trials are necessary to confirm these findings and to better understand the long-term efficacy and safety of ablation compared to cystectomy.

INTRODUCTION

Endometriosis, a condition affecting about 10% of women of childbearing age, involves the displacement of endometrial tissue outside the uterus [1, 2]. Ovarian endometriosis, also known as endometriomas, is its most frequent presentation and involves several therapeutic approaches. While

laparoscopic cystectomy, also called stripping, is the current standard, surgery can cause damage to ovarian tissue, diminishing its endocrinological and reproductive potential [3]. Various approaches have been studied to minimize this risk [4]. Among these, ablation, which involves destroying the endometriosis cells by applying energy from different sources, but which has in common the thermal damage done to the endometrioma, has found increasing use in recent years [5]. Although it is now considered an alternative method to laparoscopic stripping, the scarcity of prospective studies raises questions about its efficacy in terms of risk of disease recurrence. Recently, a meta-analysis has shown its minor impact on ovarian function [6].
In contrast, solid data on its efficacy over time in controlling the development of new endometriomas are lacking in the literature. Moreover, the lack of standardization of the technique may make it even more challenging to understand its efficacy fully. This is why we wanted to collect all the data to date in the literature on this topic. This systematic review and meta-analysis aim to assess ablation's recurrence outcomes compared to standard cystectomy for ovarian endometriomas.

MATERIALS AND METHODS

The methods for this study were specified a priori based on the recommendations in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [9]. We registered the Review for meta-analysis on the PROSPERO site with protocol number CRD549177.

Search Method

We performed systematic research for records about the use of sclerotherapy in man-aging ovarian endometriomas in PubMed, EMBASE, Scopus, Google Scholar, Clinical-trials.gov, and the Cochrane Central Register of Controlled Trials in May 2024. We did not restrict country or year of publication and considered only entirely English-published studies. We adopted the following string of idioms in each database to identify studies fitting to our review's topic: "Endometriosis and Ablation".

Study Selection

Study selection was made independently by G.A. and M.G.V. In case of discrepancy, C.R. decided on inclusion or exclusion. Inclusion criteria were: 1) studies that included patients with at least one ovarian endometrioma, treated with ablation and/or cystectomy; 2) studies reporting the outcome of interest: Recurrence Rate (RR); 3) Studies with at least 12 months of follow-up; 4) peer-reviewed articles, published originally. We excluded non-original studies, pre-clinical trials, animal trials, abstract-only publications, and articles in languages other than English. If possible, the authors of studies that were published as conference abstracts were tried to be contacted via e-mail and asked to provide their data. We mentioned the studies selected and all reasons for exclusion in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart (**Figure 1**). We assessed all included studies regarding potential conflicts of interest.

Statistical analysis

Heterogeneity among the studies was tested using the Chi-square test and I-square tests [7]. The Odds Ratio (OR) and 95% confidence intervals (CI) were used for dichotomous variables. Fixed-effect models conducted statistical analysis without significant heterogeneity ($I^2 < 50\%$), or random-effect models if $I^2 > 50\%$. Recurrence rate (RR) was used as clinical outcomes. In each study, RR was defined as the percentage of recurrence till the last follow-up. Chi-square tests were used to compare continuous variables. Review Manager version 5.4.1 (REVman 5.4.1) and IBM Statistical Package for Social Science (IBM SPSS version 25.0) for MAC were used for statistic calculation. For all performed analyses, a P-value < 0.05 was considered significant.

Quality assessment

We assessed the quality of the included studies using the Newcastle-Ottawa scale (NOS) [8]. This assessment scale uses three broad factors (selection, comparability, and exposure), with the scores ranging from 0 (lowest quality) to 8 (best quality). Two authors (CR and II) independently rated the study's quality. Any disagreement was subsequently resolved by discussion or consultation with PDF. We reported NOS Scale in **Appendix 1**.
We used a funnel plot analysis to assess publication bias. We used Egger's regression test to determine the asymmetry of funnel plots (**Appendix 2**).

Risk of Bias

The RCTs and prospective cohort studies were separately assessed, and the risk of bias in these studies was low or moderate. Saito *et al*. [11] only in-

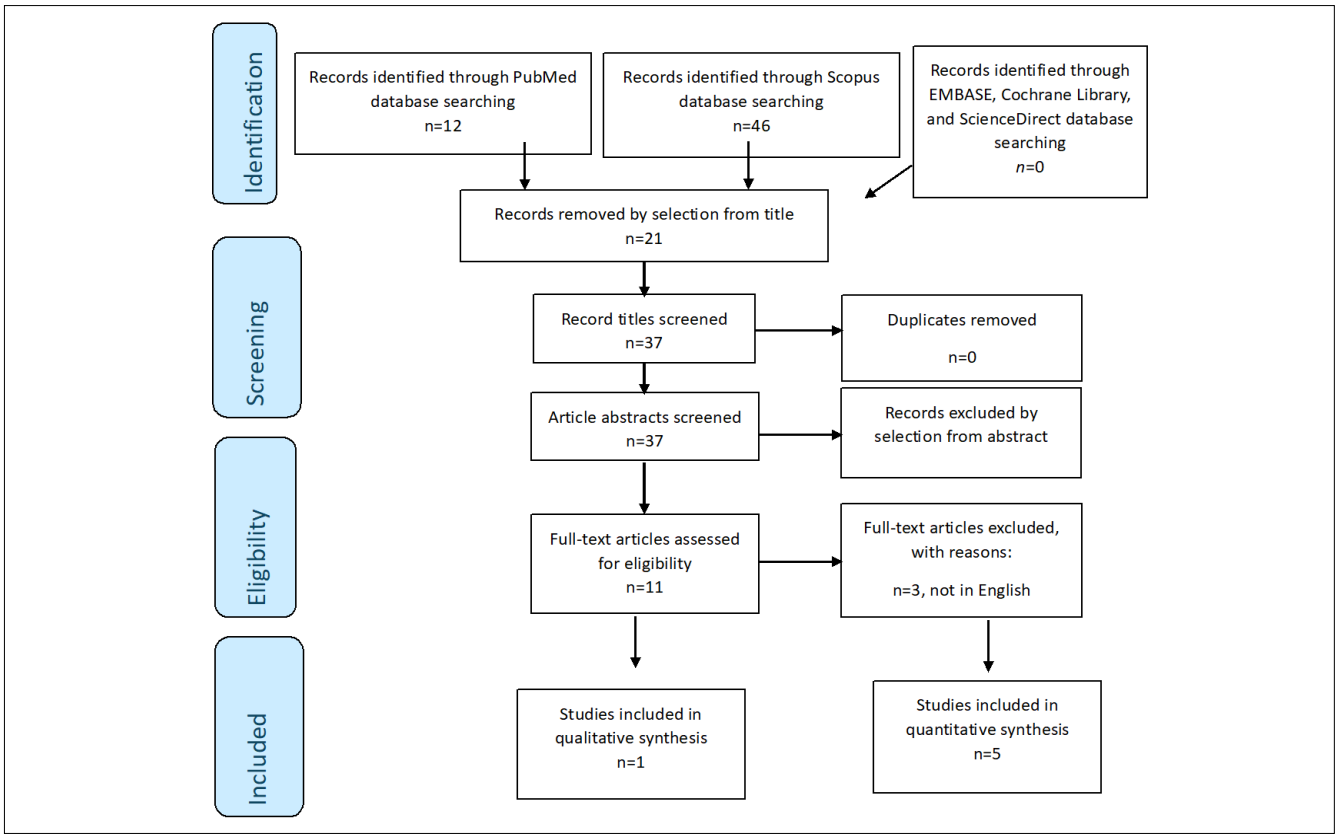

Figure 1. *Flowchart.*

cluded nulliparae in the ablation group and reported bigger dimensions of endometrioma in the ablation group. In addition, he has the shortest follow-up and has not reported any recurrence events in either group during these twelve months. Haghgoo *et al*. included 30 patients with unilateral endometrioma and 30 with bilateral endometrioma [9]. Candiani *et al*. used a CO_2 fibre laser for cyst vaporization [12], whereas argon is employed commonly.

RESULTS

Studies' Characteristics

After the database search, 58 articles matched the search criteria. After removing records with no full text, duplicates, and wrong study designs (*e.g.*, reviews), 16 were eligible. 5 matched the inclusion criteria and were included in the systematic review. 4 were comparative studies between the Ablation technique and laparoscopic stripping and were included in quantitative analysis (**Figure 1**). The countries where the studies were conducted, the publication year range, the studies' design, follow-up months, and the number of participants are summarized in **Table 1**.

NOS [8] (**Appendix 1**) assessed the quality of all studies. Overall, the publication years ranged from 2011 to 2021. In total, 395 patients with endometrioma were enrolled: 215 were treated with ablation and 180 with laparoscopic stripping.

Table 1. *Characteristics of included studies.*

Author, year of publication	Country	Period of enrollment	Study design	No. of participants	Ablation	Cystectomy
Haghgoo *et al.* 2021 [9]	Iran	2017-2019	Prospective Monocenter Cohort	58	0	58
Carmona *et al.* 2011 [10]	Spain	N/A	Prospective Monocenter Randomized	74	38	36
Saito *et al.* 2017 [11]	Japan	2011-2013	Prospective Monocenter Cohort	62	28	34
Candiani *et al.* 2019 [12]	Italy	2015-2018	Prospective Monocenter Cohort	125	61	64
Chen *et al.* 2021 [13]	China	2016	Retrospective Monocenter Cohort	76	30	46

Table 2. *Outcomes.*

Author, year of publication	Cystectomy RR (%)	Ablation RR (%)	P-value	Median FU period (months)
Single-arm studies				
Haghgoo *et al.* 2021 [9]	-	0.0	N/A	15.0
Carmona *et al.* 2011 [10]	22.0	37.0	0.4	64.0
Saito *et al.* 2017 [11]	0.0	0.0	N/A	12.0
Candiani *et al.* 2019 [12]	6.3	4.9	0.74	29.0
Chen *et al.* 2021 [13]	4.4	16.7	0.11	31.38

RR: recurrence rate; FU: follow-up.

Outcomes

The review included 395 patients. All 5 selected studies presented RR data. Overall, the RR ranged from 0 to 37.7% in the ablation group and from 0 to 22.0% in the stripping group. The follow-up period ranged from 12 to 64 months on average. Those results are summarized in **Table 2**.

By alphabetic, Candiani *et al*. [12] reported a RR of 6.3 *vs* 4.9 for ablation compared to stripping in 29 months of follow-up (p = 0.74). Carmona *et al*. [10] reported the oldest series with the highest RR in both arms (22% *vs* 37%, p = 0.4) and longest follow-up (64 months). On the contrary, Chen *et al*. [13] is the newest one, with RR 4.4% *vs* 16.7% (p = 0.11) and 31 months of observation. Haghgoo *et al*. [9] reported the only single-arm trial with no recurrence reported after 15 months. Finally, Saito *et al*. [11] did not observed recurrence in both arms.

Ablation Procedure

Haghgoo *et al.* avoided hot energy devices, as cautery, on ovaries for ablation [9]. In Carmona *et al*.'s study, the vaporization of the cyst's internal wall was performed through CO_2 laser at 30 W/cm² power density [10]. Saito *et al.* performed vaporization using bipolar current forceps (35 W) on the internal wall [11]. Candiani *et al.* used a CO_2 fibre laser in a "one-step" procedure [12]. In Chen *et al.* study, bipolar forceps were applied on the internal wall at 30W until the colour of the cyst turned white [13]. The average duration of contact between the forceps and the lesion was approximately 1 second [13]. In all cases, a biopsy was performed before proceeding with vaporization [9-13].

Meta-analysis

The 4 studies comparing ablation and stripping were enrolled in the meta-analysis. A total of 337 patients were analysed. 157 patients in the ablation arm were compared with 180 patients who underwent cystectomy, exploring RR outcome. 23 recurrences occurred in the ablation group and 14 in the stripping. Because of low heterogeneity ($I^2 = 0\%$; p = 0.45), the fixed-effects model was applied. The Cystectomy group showed a slightly non-significant higher risk for recurrence than the ablation arm (RR 1.99 [95%CI 0.95-4.16] p = 0.07) (**Figure 2**).

DISCUSSION

Main Findings

This systematic review and meta-analysis included five studies, four cohort studies, and one randomized trial. The qualitative data analysis could not show a statistically significant difference between the ablation technique and laparoscopic stripping based on recurrence (p = 0.07). However, stripping

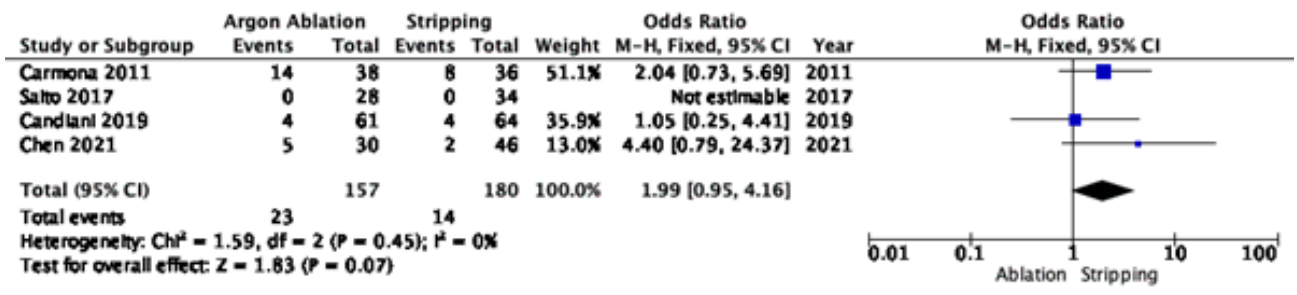

Figure 2. *Forest Plot.*

seems to show a worsening trend with an OR per recurrence of 1.99 and a 95%CI slightly including neutrality (0.95-4.16). This could be confirmed as the sample size increases. Moreover, the largest weighting (51.1%) is represented by Carmona *et al*. [10] which has the advantage of being the only randomised clinical trial and the disadvantage of being the oldest included study, risking being flawed by a technological backwardness, which may undermine the efficacy of the ablative technique.

Comparison with existing literature

Since its first publication, ablation has been an attractive alternative for treating endometriomas. This is because the relationship between endometriosis and infertility is an intrinsic one, worsened by all treatment episodes of a surgical nature. Cyst removal inevitably results in a reduction in the patient's reproductive and endocrinological potential. Much scientific society has struggled to minimize this impact, on the one hand, by attempting to optimize post-stripping coagulation techniques [3] and, on the other hand, in seeking alternative approaches for treating endometrioma, such as sclerotherapy [4]. In the same vein, all alternatives provided to stripping, including ablation, have always been weighed first in assessing their impact on fertility. Recently, Zhang *et al*. [5] published a meta-analysis on pre- and post-operative differences in AMH and Antral Follicle Count (AFC) determined by ablation and stripping. The 294 patients enrolled in this meta-analysis showed a lower AFC in the stripping arm, both in the immediate postoperative period (mean differences [MD], 1.33; 95% credible interval, 2.15 to 0.51; I^2 1/4 57%), and at 6-month follow-up (MD, 1.93; 95% credible interval, 2.40 to 1.45; I^2 1/4 0%). The intragroup comparisons of AMH levels supported negative effects on ovarian reserve of both cystectomy (MD, 1.26; 95% credible interval, 1.64 to 0.88; I^2 1/4 45%) and ablation (MD 0.70; 95% credible interval, 1.07 to 0.32; I^2 1/4 0%). These data support the evidence from another systematic review on the use of cystectomy [14] and a meta-analysis [15] conducted by two independent groups. A group in our analysis also published data from the same series on this subject, with a more significant impact of cystectomy on AMH and AFC [16]. On the other hand, much rarer are the papers that focus on ablation's efficacy regarding recurrence risk. In this scenario, to our knowledge, our meta-analysis stands as the only meta-analysis focusing on this topic.

Clinical Implication

Once the lower impact on patients' fertility has been established, it is also essential to weigh up the effectiveness of the ablative technique over time. This is why, in our opinion, the evidence derived from our work supports the therapeutic choices for treating patients with endometriosis. Endometriosis is a chronic condition where gaining time between treatment episodes is crucial to optimizing treatment. To date, surgery remains the diagnostic gold standard, but this contrasts with the need to be minimally invasive. This discrepancy often creates diagnostic delays that condition the severity of the clinical presentation [17]. On the one hand, we need to optimize diagnosis by identifying biomarkers, such as liquid biopsy [18], that can intercept our patients before organic progression, and on the other hand, we need to improve our therapeutic options to chronicle the disease with as little morbidity as possible. Fortunately, the pharmacological landscape has recently expanded with new drugs such as Relugolix, which have shown promising results in controlling symptoms [19]. Surgery should go hand in hand with adapting to the growing number of therapeutic options. In this scenario, we believe standardization of the ablative technique would also be fundamental to improve its reproducibility.

Strengths and Limitations

Our study is the first meta-analysis to evaluate whether cystectomy or ablation results in higher endometrioma RR. Unfortunately, very few studies on this subject limit our case series to 395. Our results were not statistically significant, even though they showed a clear trend against cystectomy. Another limitation is the lack of standardization in ablative techniques and haemostatic approaches in the case of stripping. This attempt to compare the two methods is more ambitious.

Furthermore, studies that have used bipolar coagulation have not reported any data on its intensity. A further limitation is the possibility of bilateral neoformations, which were included in the studies as a single patient, even though it is assumed that the double procedure exposes one to a double risk of recurrence. Finally, no data have been reported regarding spillage during treatment, which may promote intraabdominal spillage of endometriosis tissue and increase the chances of recurrence [20, 21].

Finally, a final point should be made that our review does not take into account additional outcomes that may differentiate the two techniques, such

as the risk of postoperative pain [22] or the risk of malignant transformation [23].

CONCLUSIONS

Our study failed to show a statistically significant ($p = 0.07$) increased safety profile of ablation compared to cystectomy in terms of RR. However, the data show a clear trend with almost doubled risk of recurrence in patients undergoing laparoscopic cystectomy (OR 1.99, 95%CI 0.95-4.16). Further randomised trials may support or refute this trend.

COMPLIANCE WITH ETHICAL STANDARDS

Authors' contributions
C.R.: Conceptualization, formal analysis, methodology, project administration, software, supervision, validation, writing - original draft, writing - review & editing. I.I.: Data curation, writing - review & editing. M.G.V: Data curation, investigation, project administration, software, supervision. C.V.: Data curation, investigation. G.A.: Data curation, formal analysis. M.T.: Supervision, validation, visualization. P.dF.: Supervision, validation, visualization.

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Study registration
We registered the Review for meta-analysis on the PROSPERO site with protocol number CRD549177.

Disclosure of interests
The authors declare that they have no conflict of interests.

Ethical approval
N/A.

Informed consent
N/A.

Data sharing
Data are available along with the article.

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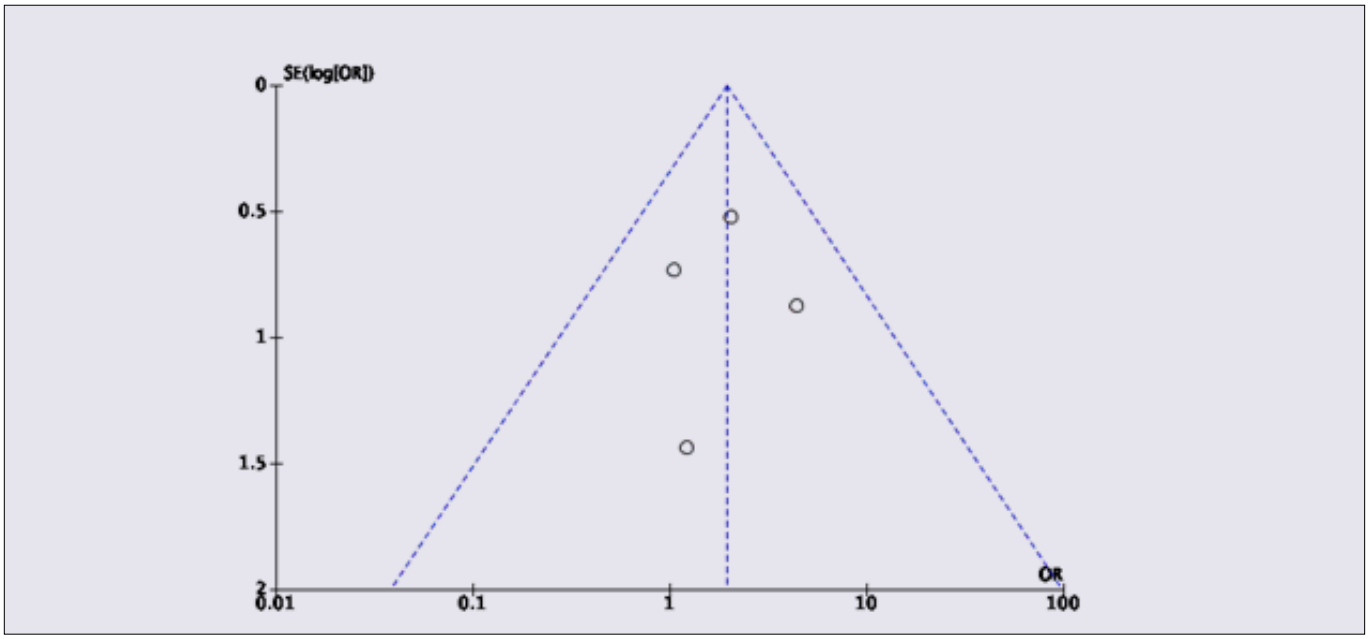
SUPPLEMENTS

Appendix 1. *Newcastle-Ottawa Scale.*

Author, year of publication	Country	Selection	Comparability	Exposure	Total
Single-arm studies					
Haghgoo *et al.* 2021	Iran	3	1	1	5
Carmona *et al.* 2011	Italy	3	2	2	7
Saito *et al.* 2017	Japan	2	1	2	5
Candiani *et al.* 2019	Italy	2	1	3	6
Chen *et al.* 2021	China	2	2	3	7



Appendix 2. *Funnel Plot.*

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Sexual education and adolescents: a systematic review and meta-analysis

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ABSTRACT

Objective. Sexuality plays a significant role in human mental and physical health, well-being, and overall life satisfaction. Unfortunately, misinformation and lack of awareness remain barriers to access essential healthcare for adolescents. As a result of numerous scientific studies, many experts have reached the consensus that maintaining adolescents' sexual health requires comprehensive and scientifically accurate education and healthcare services. Therefore, the purpose of this systematic review and meta-analysis was to evaluate the impact of sexual education on the sexual health of adolescents.

Materials and Methods. The search was conducted in electronic databases including PubMed, Scopus, the Cochrane Library, Google Scholar, and ClinicalTrials.gov. 9 research articles, resulting in a total of 17,667 patients, that evaluated the impact of sexual education on the sexual knowledge of adolescent girls and boys, sexual initiation, unwanted pregnancy, abortions, and sexually transmitted diseases were included in this systematic review.

Results. The analysis of included studies presented that sexual education improves knowledge of contraception methods among adolescents ($p < 0.00001$) and the rate of unwanted pregnancies ($p = 0.02$), but no significant difference was obtained assessing the level of contraception use ($p = 0.39$) and the sexual experience ($p = 0.11$).

Conclusions. According to the results of our systematic review and meta-analysis, we emphasize the importance of sexual education and healthy lifestyle promotion in families and schools in early adolescence to improve adolescents' knowledge of contraceptive methods and reduce the incidence of unwanted pregnancies. However, further research is needed to gain a deeper understanding of the topic.

INTRODUCTION

Sexuality is an essential aspect of our lives, playing a significant role in human health and well-being. Modern scientific data proves that the sexual sphere directly affects mental and physical health and therefore overall life satisfaction. It was presented that sexual dysfunction is associated with several

general health comorbidities, which makes it vital to maintain sexual health [1].

Unfortunately, lack of quality sexual education, created by social and cultural norms, stigmatizes this area of our lives and directly contributes to poor sexual health outcomes. Social pressure, which leads to stigma, has been shown to increase emotional stress, resulting in a negative impact on mental condition and overall quality of life [2].

Adolescence is an important life stage that is characterized by significant physical growth and mental and social development. This period of development has a direct impact on the formation of an individual's personality in adulthood and lays the foundation for their reproductive and overall health [3]. Additionally, it is important to acknowledge that during this phase, a teenager may begin participating in sexual activities and experimentation. Early sexual initiation (ESI), typically defined as the first sexual experience occurring at or before the age of 15, is currently a common occurrence [4]. Scientific data from Canada provides that 30% of adolescents aged 15 to 17 years had intercourse at least once, whereas 10% of males and 8% of females report first genital intercourse occurring before age 15 years [5]. According to the US data, the early sexual debut was presented for 27.3% among boys and 20.7% among girls [6].

ESI is associated with risky behaviours such as unprotected intercourse or inconsistent contraceptive use, multiple sexual partners, and drug use [4]. These factors adversely affect adolescent sexual and reproductive health (SRH), increasing the risk of sexually transmitted diseases (STDs), unplanned pregnancies, abortions, and teen dating violence. SRH is an integral part of care for all adolescents, including those with special needs and chronic health conditions [5, 6].

Misinformation and lack of awareness remain barriers to access essential healthcare for adolescents. As a result of numerous scientific studies, many experts have reached the consensus that maintaining adolescents' sexual health requires comprehensive and scientifically accurate education and healthcare services [2, 3, 7].

Sexual education (SE) has a huge number of areas, aimed at addressing relevant issues in the field of adolescent sexual health. For example, the U.S. Centers for Disease Control and Prevention (CDC's) framework for SRH has been adapted to 7 topics: Partners, Practices, Protection from sexually transmitted infections (STIs), Past history of STIs, Prevention of pregnancy, Permission (consent), and Personal (gender) identity [5].

First, it is important to deal with the spread of sexually transmitted infections (STIs) among adolescents. Complications of untreated STIs should not be ignored; they include pelvic inflammatory diseases, chronic pelvic pain, infertility, and foetus or neonate pathologies. Complicated outcomes of untreated STIs, such as inflammatory conditions of the pelvis, chronic pelvic discomfort, infertility, and foetal or newborn pathologies, must not be disregarded. Currently, it has been demonstrated that the incidence of the human immunodeficiency virus (HIV) among young individuals is significantly influenced by the age of sexual debut. Adolescents who have experienced ESI are also at increased risk of human papillomavirus (HPV) infection due to the rapid physiologic changes and immature immune responses [4, 8]. This aspect requires special vigilance due to the social nature of this disease and the immediate threat of malignant neoplasms in infected individuals [9, 10]. Secondly, SE helps to address the issue of unwanted teenage pregnancies and abortions. Approximately 77% of adolescent pregnancies are unplanned, leading to abortions or abandonment of babies [7, 11, 12]. It has been noted that teenage mothers are at a higher risk of experiencing postpartum depression, dropping out of school, and living in poverty. This highlights the ongoing need for effective contraceptive education among adolescents [4, 13]. It is equally important to pay attention to the role of sexual education in ensuring reliable contraception for patients carrying BRCA1/2 genes, as there are a number of myths and misunderstandings that negatively affect the quality of life of such patients [14].

Despite the existing data, there is a lack of research that fully demonstrates the benefits of any particular type of sex education. A notable example is school-based health care (SBHC) programs, which provide accessible sexual, reproductive, and mental health services tailored to the needs of adolescents. The World Health Organization defines comprehensive sexuality education as providing accurate, age-appropriate information on sexuality and sexual and reproductive health [11, 15].

After carefully reviewing the available literature, we have selected this model of sexual education for evaluating in our systematic review and meta-analysis.

Despite the numerous aspects that comprise the topic of sex education, we have chosen to focus on

the effectiveness of school-based sexual education on the onset of sexual activity, sexual experience, the use of contraceptive methods, pregnancy rate, the prevention of unwanted pregnancies, abortions, and sexually transmitted diseases of adolescents.

METHODS

Study design and registration

The present systematic review included all published research articles that evaluated trials aimed at elucidating the impact of sexual education on sexual knowledge in adolescent girls and boys, sexual initiation, unwanted pregnancy, abortions, and sexually transmitted diseases. Our systematic review was conducted according to the PRISMA 2020 checklist [16].

Institutional Review Board (IRB) approval was not requested as this study is a review of published studies. The present systematic review has been registered in the PROSPERO international prospective register of systematic reviews by the National Institute for Health Research (NIHR). The registration number is PROSPERO 2022 CRD42022357877 [17].

Search strategy

The search was conducted independently by five investigators (J.A., E.K., Y.D., K.K., E.Z.) in PubMed, Scopus, the Cochrane Library, Google Scholar, and ClinicalTrials.gov from August 2023 to October 2023 to identify studies that reported the impact of sexual education on the sexual knowledge of adolescents, unwanted pregnancy, abortions, and sexually transmitted diseases, pregnancy rate, sexual initiation, incidence of STDs, contraceptive use, abortion rates, and knowledge of STDs, pregnancy, and contraceptive methods. The search consisted of controlled terms (*e.g.*, MESH) and text words for sex education, reproductive health, pregnancy, gynaecology, contraceptive methods, adolescents, contraception, girls, STDs, sexually transmitted diseases, and abortion. The retrieved records were imported in ZOTERO, and duplicate records were removed. Cited and citing references of the included studies were screened for additional relevant publications.

Inclusion criteria

We included randomized clinical trials (RCTs) and nonrandomized clinical trials (prospective controlled, prospective cohort, retrospective studies, and other types of studies) that included a minimum of 10 patients and were school-based. Intervention studies, animal studies, conference abstracts, and studies in languages other than English were excluded.

Study selection and data extraction

Five investigators (J.A., E.K., Y.D., K.K., E.Z.) independently read the full texts of the preselected articles to verify the eligibility of the articles based on their titles and abstracts with the use of COVIDENCE. After this step, studies were excluded if there were duplicate datasets. Any disagreements regarding the inclusion or exclusion of preselected studies and any other disagreements during the review process were resolved with the help of the sixth author (L.P.).

The included studies were independently collected by five authors (J.A., E.K., Y.D., K.K., E.Z.) using a standardized data extraction procedure (authors, publication year, study design, patient characteristics, intervention, and outcomes).

Quality assessment

Risk-of-bias assessment was conducted for each of the studies included using the Cochrane Handbook for Systematic Reviews of Interventions [18]. Five investigators (J.A., E.K., Y.D., K.K., E.Z.) independently assessed the quality of the selected studies. A fourth investigator (L.P.) was involved when disagreements occurred. Following the Cochrane Handbook for Systematic Reviews of Interventions, the RoB2 tool [19] was used to assess the risk of bias in randomized-controlled studies, and ROBINS-I [20] was used for non-randomized studies (prospective controlled, prospective cohort, retrospective studies, and other types of studies). These tools were also used to assess the risk of bias arising from reporting biases due to missing results in synthesis. The quality of evidence (QoE) was assessed according to the GRADE system [21].

Data synthesis

All statistical analyses were performed with Review Manager (RevMan 5.4) (recommended by the Cochrane Society).

RESULTS

Study selection

The whole search strategy with the results is presented in the PRISMA flow diagram (**Figure 1**).

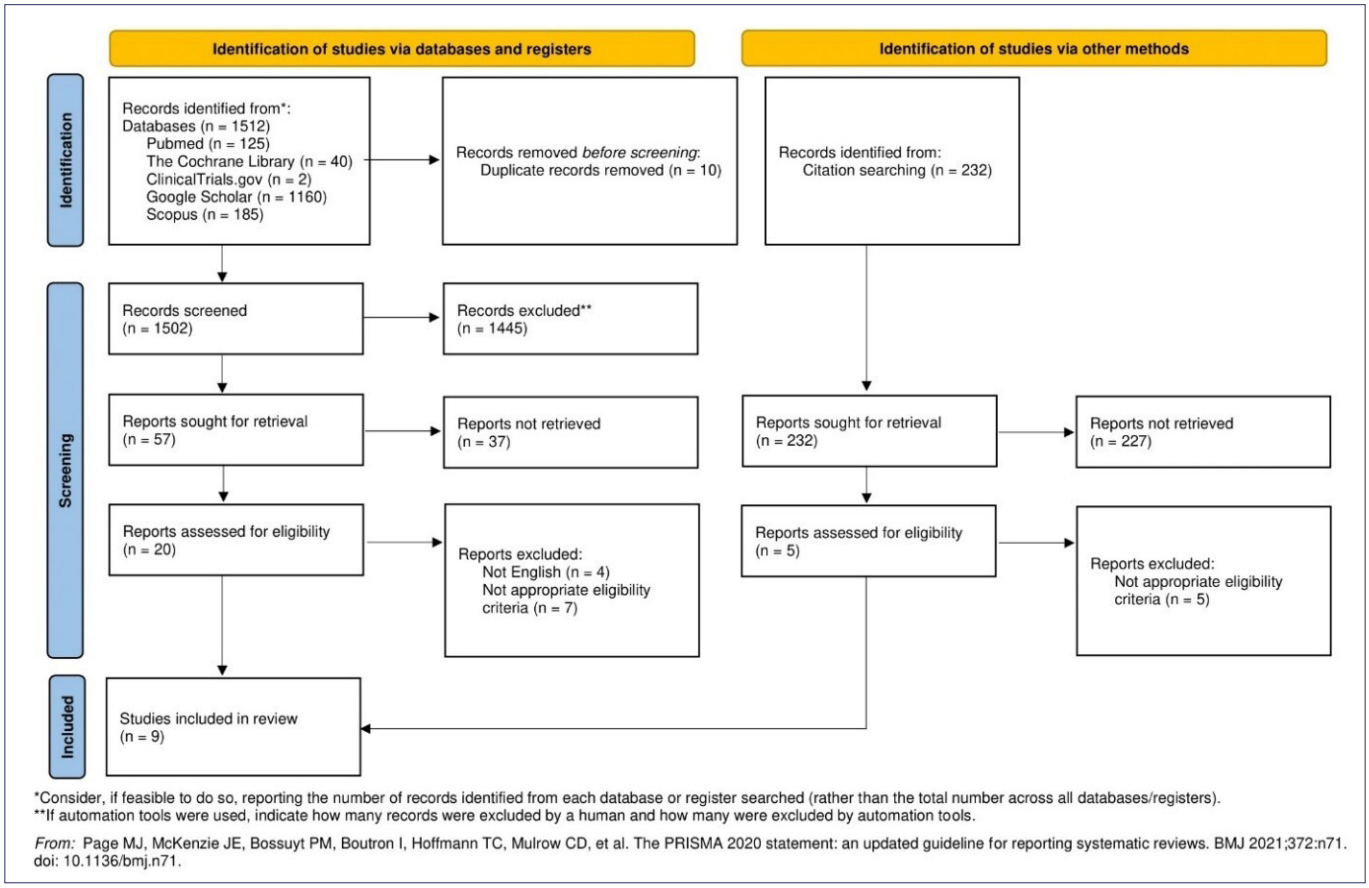

Figure 1. *Flow diagram of the literature search and study selection process according to the PRISMA guidelines.*

We used an electronic search of PubMed, Scopus, Google Scholar, Clinical Trials, and the Cochrane Library databases and received 1512 articles. After removing duplicates and searching for the title and abstract of the articles, 57 publications were selected. Of these, 37 articles were excluded after reading the full texts. Four studies were not in English [22-25]. Also, due to the absence of relevant inclusion criteria, 11 studies were excluded. 4 of them were not in English, and others were without appropriate inclusion criteria (**Table 1**).
Furthermore, we checked the references of the selected articles for acceptable studies (n = 232).

Table 1. *The list of excluded studies.*

n	Studies	Reasons for exclusion
1	Walker *et al.*, 2006 [25]	Absence of appropriate outcomes.
2	Kraft *et al.*, 2012 [41]	Absence of appropriate outcomes.
3	Constantine *et al.*, 2015 [42]	Absence of appropriate outcomes.
4	Li *et al.*, 2017 [39]	Absence of appropriate outcomes.
5	Netshikweta *et al.*, 2018 [43]	Absence of appropriate outcomes.
6	Dongarwar *et al.*, 2019 [44]	Absence of appropriate outcomes.
7	Marsiglio *et al.*, 1986 [45]	Absence of appropriate outcomes.

We selected 5 articles for full-text review, but all of them had inappropriate eligibility criteria.

Included studies

Therefore, a total of 9 studies were included in the qualitative synthesis of the systematic review [26-34], resulting in a total number of patients that is less in cohorts due to incomplete follow-up. Four studies were comparative [26, 28, 31, 34], three cluster randomized [30, 32, 33], one was an educational intervention [27] and one was prospective [29] (**Table 2**).

Type of intervention

The studies that investigated and compared the impact of sexual education on the sexual health of adolescents. The content of school-based sexual education programs of articles included in the systematic review is presented (**Table 3**).

Type of patients

A group of adolescents received school-based sexual education programs (n = 9,346) and a group of adolescents didn't receive school-based sexual education programs (n = 8,825) (17,667 patients totally).

Table 2. *Description of articles included in the systematic review.*

Study (first author)	Type of study	Participants/ population	Intervention(s), exposure(s)	Comparison(s)/ control	Outcome(s)
Knowledge of contraception methods					
G. Bogani 2017 [26]	Comparative Study	664 adolescents (median age 14 years)	First group: School-based sexual education programs (n = 559)	Second group: Without education (n = 105)	First group: 322 (58%) Second group: 42 (40%) (p<0.01)
A. M. Morenz 2019 [29]	Prospective study	520 female and male students (mean age 17.5)	Post-test (n = 503) After 2-h sexual education	Pre-test (n = 520) Before 2-h sexual education	Post-test n = 429 (85%) Pre-test n = 312 (60%) (p < 0.01)
S. R. Tortolero 2010 [30]	Cluster randomized study	907 adolescents (mean age 13.2 vs 13.0 years; data not shown)	First group: adolescents who received sex education (n = 349)	Second group: adolescents who didn't receive sex education (n = 558)	First group: 1.76±1.01 Second group: 1.64±1.03
Use of contraceptives					
G. Bogani 2017 [26]	Comparative Study	664 adolescents (median age 14 years)	First group: School-based sexual education programs (n = 559)	Second group: Without education (n = 105)	First group from 133: • Condom 73 (55%), • Hormonal contraception 20 (15%) Second group from 31: • Condom 17 (55%), • Hormonal contraception 5 (16%) (p = 1.00)
S. P. de Weiss 1991[32]	Cluster randomised trial	392 adolescents (12-19 years)	First group: sexual education (n = 211)	Second group: no education (n = 178)	First group: 31 (62%) from 50 Second group: 28 (62.3%) from 45
J. Stephenson 2008 [33]	Cluster randomised trial	Pupils (n = 8,766)	First group: usual teacher-led sex education (n = 4 250)	Second group: peer-led sex education (n = 4 516)	Girls: First group: 854 (86.35%) from 989 Second group: 631 (84.47%) from 747 Boys: First group: 650 (87.01%) from 747 Second group: 509 (87.76 %) from 580
M. Taylor 2014 [34]	Comparative Study	679 adolescent (15-19 years)	First group: adolescents who received sex education (n = 383)	Second group: adolescents who didn't receive sex education (n = 296)	First group: 39 (54.2%) from 72 Second group: 11 (36.7%) from 30 (p < 0.01)
B. Wang 2008 [31]	Comparative Study	2,227 participate (15-24 years)	First group: Post-test (n = 1,148) -boys- 678 -girls- 470	Second group: Pre-test (n = 1,148) -boys- 678 -girls- 470	Boys Pre-test: 3,5 from 12 Post-test: 8,4 from 12 Girls Pre-test: 2,6 from 12 Post-test: 8,2 from 12 (p < 0.001)
Unwanted pregnancy rate					
P. K. Kohler 2007 [27]	Comparative Study	1,719 adolescents (15-19 years): - girls 47.4% - boys 52.6%	First group: adolescents who received comprehensive sex education (n = 1,161) Third group: adolescents who received abstinence-only education (n = 390	Second group: adolescents who didn't receive sex education (n = 168)	Pregnancy rate (Weighted% (95%CI)) First group: 53.5 (42.3–64.5) Second group: 19.4 (13.2–27.4) Third group: 27.1 (17.7–39.1)
D. Malovizky 1997 [28]	Educational intervention study	1,793 women (close to the age of 18 years)	First group: adolescents who received comprehensive sex education (n = 968)	Second group: adolescents who didn't receive sex education (n = 825)	First group: 25 (2.6%) Second group: 33 (4%)
J. Stephenson 2008 [33]	Cluster randomised trial	Pupils (n = 8,766)	First group: usual teacher-led sex education (n = 4,250)	Second group: peer-led sex education (n = 4,516)	First group: 103 (7.65%) from 1,346 Second group: 93 (9.96%) from 934

Study (first author)	Type of study	Participants/ population	Intervention(s), exposure(s)	Comparison(s)/ control	Outcome(s)
M. Taylor 2014 [34]	Comparative Study	679 adolescent (15-19 years)	First group: adolescents who received sex education (n = 383)	Second group: adolescents who didn't receive sex education (n = 296)	First group: 5 (6.3%) from 80 Second group: 2 (4.4%) from 46
Sexual experience					
P. K. Kohler 2007 [27]	Comparative Study	1,719 adolescents (15-19 years):	First group: adolescents who received comprehensive sex education (n = 1161) Third group: adolescents who received abstinence-only education (n = 390)	Second group: adolescents who didn't receive sex education (n = 168	Weighted % (95% CI)) First group: 66.0 (61.6–70.2) Second group: 11.5 (8.9-14.7) Third group: 22.6 (19.1- 26.5) (p = 0.06)
S. P. de Weiss 1991 [32]	Cluster randomised trial	392 adolescents (12-19 years)	First group: sexual education (n = 211)	Second group: no education (n = 178)	First group: 50 (23.69%) from 211 Second group: 45 (25.28%) from 178
M. Taylor 2014 [34]	Comparative Study	679 adolescent (15-19 years)	First group: adolescents who received sex education (n = 383)	Second group: adolescents who didn't receive sex education (n = 296)	First group: 82 (21.4%) Second group: 47 (15.9%)
S. R. Tortolero 2010 [30]	Cluster randomised trial	907 adolescents (mean age 13.2 vs 13.0 years; data not shown)	First group: adolescents who received sex education (n = 349)	Second group: adolescents who didn't receive sex education (n = 558)	First group: 37 (10,6%) Second group: 46(8,3%)
Knowledge of HIV/STD transmission					
G. Bogani 2017 [26]	Comparative Study	664 adolescents (median age 14 years)	First group: School-based sexual education programs (n = 559)	Second group: Without education (n = 105)	First group: 458 (82%) Second group: 75 (71%) (p = 0.01)
S. R. Tortolero 2010 [30]	Cluster randomised trial	907 adolescents (mean age 13.2 vs 13.0 years; data not shown)	First group: adolescents who received sex education (n = 349)	Second group: adolescents who didn't receive sex education (n = 558)	First group: 0.57±0.31 Second group: 0.55±0.29
B. Wang 2008 [31]	Comparative Study	2,227 participate (15-24 years)	First group: Post-test (n = 1,148) -boys- 678 -girls- 470	Second group: Pre-test (n = 1148) -boys- 678 -girls- 470	Boys Pre-test: 5,7 from 12 Post-test: 8,1 from 12 Girls Pre-test: 5.1 from 12 Post-test: 8,1 from 12 (p < 0.001)
STD diagnosis					
P. K. Kohler 2007 [27]	Comparative Study	1,719 adolescents (15-19 years): - girls 47.4% - boys 52.6%	First group: adolescents who received comprehensive sex education (n = 1,161) Third group: adolescents who received abstinence-only education (n = 390	Second group: adolescents who didn't receive sex education (n = 168)	(Weighted % (95% CI)) First group: 73.4 (59.5–83.8) Second group: 6.9 (3.0–15.4) Third group: 19.7 (11.1–32.6) (p = 0.55)
Knowledge of pregnancy					
A. M. Morenz 2019 [29]	Prospective study	520 female and male students (mean age 17.5)	Post-test (n = 503) After 2-h sexual education	Pre-test (n = 520) Before 2-h sexual education	Post-test: n = 461 (92%) Pre-test: n = 187 (36%)
Abortion rate					
J. Stephenson 2008 [33]	Cluster randomised trial	Pupils (n = 8,766)	First group: usual teacher-led sex education (n = 4 250)	Second group: peer-led sex education (n = 4,516	Abortion rate First group: 53 (3.91%) from 1,356 Second group: 48 (5.11%) from 939

Risk of bias assessment

According to the Cochrane Handbook for Systematic Reviews of Interventions, the risk of bias of included studies was assessed independently by five reviewers (J.A., E.K., Y.D., K.K., E.Z.), using ROBINS-I [20] for non-randomized studies and RoB 2 [19] for randomized control trials. Any disagreements were resolved by discussion with the sixth author (L.P.). The ROBINS-I tool included bias due to confounding, bias in the selection of study participants, bias in the classification of interventions, deviations from intended interventions, bias due to missing data, bias in the measurement of outcomes, and bias in the selection of the reported results. Each of the items was classified as critical, serious, low risk of bias, or no information. The Rob 2 tool included bias arising from the randomization process due to deviations from intended interventions, missing outcome data, bias in the measurement of the outcome, and bias in the selection of the reported result. Each of the items was classified as high, of some concern, or indicating low risk of bias. Visualization tools were created by the ROBVIS app. This app created "traffic light" plots of the domain-level judgements for each result and weighted bar plots of the distribution of risk-of-bias judgments within each bias domain. The overall risk of bias for non-randomized trials was serious in 33% and moderate in 67%, according to the ROBINS-I tool, and based on the RoB 2 tool (**Figure 2**), randomized trials had 100% some concerns regarding the overall risk of bias.

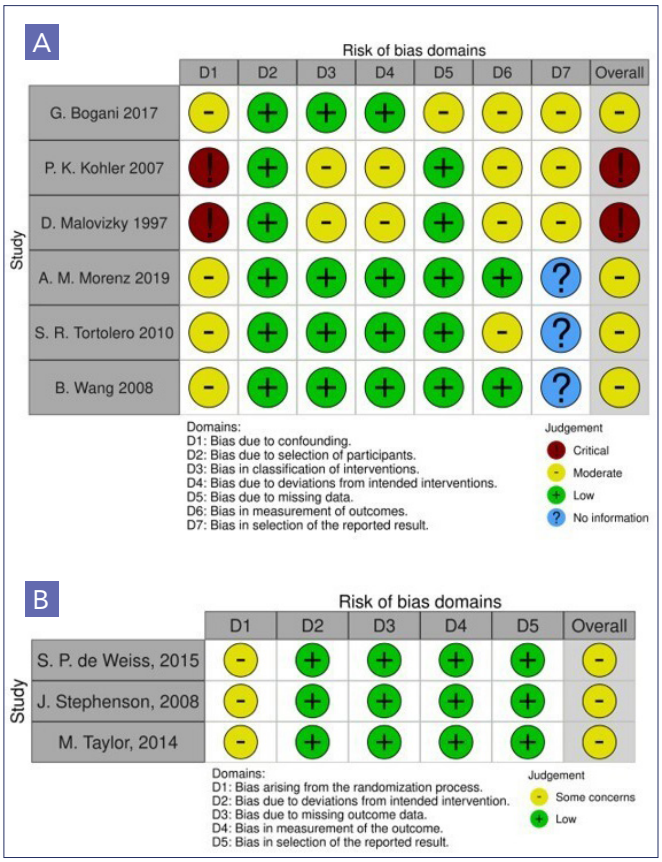

Figure 2. *(**A**) ROBINS-I tool for non- randomized trials; (**B**) RoB 2 tool for randomized trials.*

Effects of intervention

The primary outcome was the impact of sexual education on the knowledge of contraceptive methods. The secondary outcome was the impact

Table 3. *Content of school-based sexual education programs of articles included in the systematic review..*

Study (first author)	Content of school-based sexual education programs
G. Bogani 2017 [26]	Anatomy, reproduction, health, responsibilities, STDs, birth control, abstinence.
P. K. Kohler 2007 [27]	Formal sex education (abstinence-only) OR comprehensive sex education (both "saying no to sex" and birth control)
D. Malovizky 1997 [28]	Sexuality, anatomy and physiology of the reproductive system, contraceptive methods, pregnancy and childbirth, decision-making processes, interpersonal relationships, pregnancy prevention, STDs, personal hygiene
A. M. Morenz 2019 [29]	Adolescent sexuality, demographic information regarding adolescent pregnancy, complications during pregnancy, methods of birth control and protection from STD
S. R. Tortolero 2010 [30]	A life skills decision-making paradigm (Select, Detect, Protect), setting personal limits and practicing refusal skills related to sexual behavior, characteristics of healthy dating relationships; reproduction and STDs, importance of HIV, STI, and pregnancy testing if person is sexually active; skills training condom and contraceptive use; parent–child homework activities to facilitate dialogue on such topics as friendship qualities, dating, and sexual behavior
B. Wang 2008 [31]	Abstinence, sexuality, contraception, HIV/AIDS prevention, healthy sexual behaviors
S. P. de Weiss 1991 [32]	How to prevent pregnancy, where to obtain contraceptives, relationship with sex partner, sexuality
J. Stephenson 2008 [33]	Skills in sexual communication and condom use, pregnancy, STDs, contraception, local sexual health services
M. Taylor 2014 [34]	Modules: «Knowing Yourself», «The Choice is Yours», «Relationships», «Making Choices», «Body Development», «Contraception», «Peer Pressure», «Culture», «Parenthood», «Responsibility», «Human Rights», «Gender».

of sexual education on the use of contraceptives. The tertiary outcome was the impact of sexual education on unwanted pregnancies. The quaternary outcome was the impact of sexual education on the sexual experience of adolescents.

Sensitivity analysis

According to the Cochrane Handbook for Systematic Reviews of Interventions, an I^2 value of 0 indicates no observed heterogeneity, whereas I^2 values from 30% to 60% may represent moderate heterogeneity, I^2 values from 50% to 90% may represent substantial heterogeneity, and I^2 values from 75% to 100% represent considerable heterogeneity.

Knowledge of contraception methods

The level of knowledge about contraception among adolescents was assessed by three studies. Bogani *et al*.'s study [26] presented that students who received school-based sexual education have significantly better knowledge about methods of contraception than students' groups without education (58% *vs* 40% ($p < 0.01$)). The same results were demonstrated by Morenz *et al*. [29] (85% *vs* 60% ($p < 0.01$)) and Tortolero *et al*.'s [30] studies.

Also, we have done the primary analysis as a meta-analysis of two studies by Bogani *et al*. [26] and Morenz *et al*. [29] with a total of 1,184 adolescents: RR = 1.42, 95%CI 1.32 to 1.54, $p < 0.00001$. The heterogeneity for this comparison was 0%. Consequently, sex education shows better knowledge of contraception methods among adolescents who received sex education compared to adolescents who did not (**Figure 3**).

Use of contraceptives

Such an important parameter as use of contraceptives by adolescents was investigated by five studies. A study by Bogani *et al*. [26] showed that the use of condoms was the same in groups of students who received or didn't receive school-based sexual education, and the use of hormonal contraception was higher in the group without sexual education, but the difference wasn't significant. de Weiss *et al*.'s study [32] presented similar results, showing that the group without sexual education had a higher percentage of contraception using, but the difference was also insignificant. Although Taylor *et al*.'s study [34] demonstrated that the presence of sexual education had a positive effect on the test results of students (54.2% *vs* 36.7% ($p < 0.01$)). Also, the authors proved that the results of students after completing the course of sexual education were significantly better than before it (8.4 *vs* 3.5 among boys and 8.2 *vs* 2.6 among girls ($p < 0.001$)). Stephenson *et al*.'s study [33] compared use of contraceptives between students who received teacher-led education, with students who received peer-led sexual education and demonstrated that the results of the second group were better among boys, whereas among girls, the teacher-led education group showed better results.

The secondary analysis was conducted to compare the use of contraceptives. Four studies [26, 32-34] were included in the meta-analysis: RR = 1.01, 95%CI 0.98 to 1.04, $p = 0.39$. The heterogeneity for this comparison was 0%. Based on our analysis, it can be concluded that there is no significant difference in the level of contraception use by adolescents who received sex education compared to adolescents who did not (**Figure 2**).

Unwanted pregnancy rate

The percentage of unwanted pregnancies among adolescents was assessed by four studies. In Kohler *et al*.'s study [27] the group that received comprehensive sex education demonstrated the highest pregnancy rate, whereas the group that received abstinence-only sex education demonstrated a higher pregnancy rate than students without sex education. Taylor *et al*.'s study [34] showed similar results: the group that received sex education demonstrated higher pregnancy rate than students who didn't. However, in Malovizky *et al*.'s study [28], pregnancy rate of adolescents received comprehensive sex education was lower than among adolescents, didn't receive sex education. Interestingly, Stephenson *et al*.'s study [33] presented that a teacher-led sex education group of adolescents demonstrated a higher pregnancy rate than students who passed peer-led sex education.

In the third analysis, we compared the rates of unwanted pregnancy. Three studies were included in the meta-analysis [28, 33, 34]: RR = 0.75, 95%CI 0.59 to 0.95, $p = 0,02$. The heterogeneity for this comparison was 0%. Based on the data obtained, the rate of unwanted pregnancies is significantly lower in adolescents who received sexual education (**Figure 2**).

Sexual experience

The sexual experience among adolescents was also investigated. In Kohler *et al*. study [34] comprehensive sex education group demonstrated the most

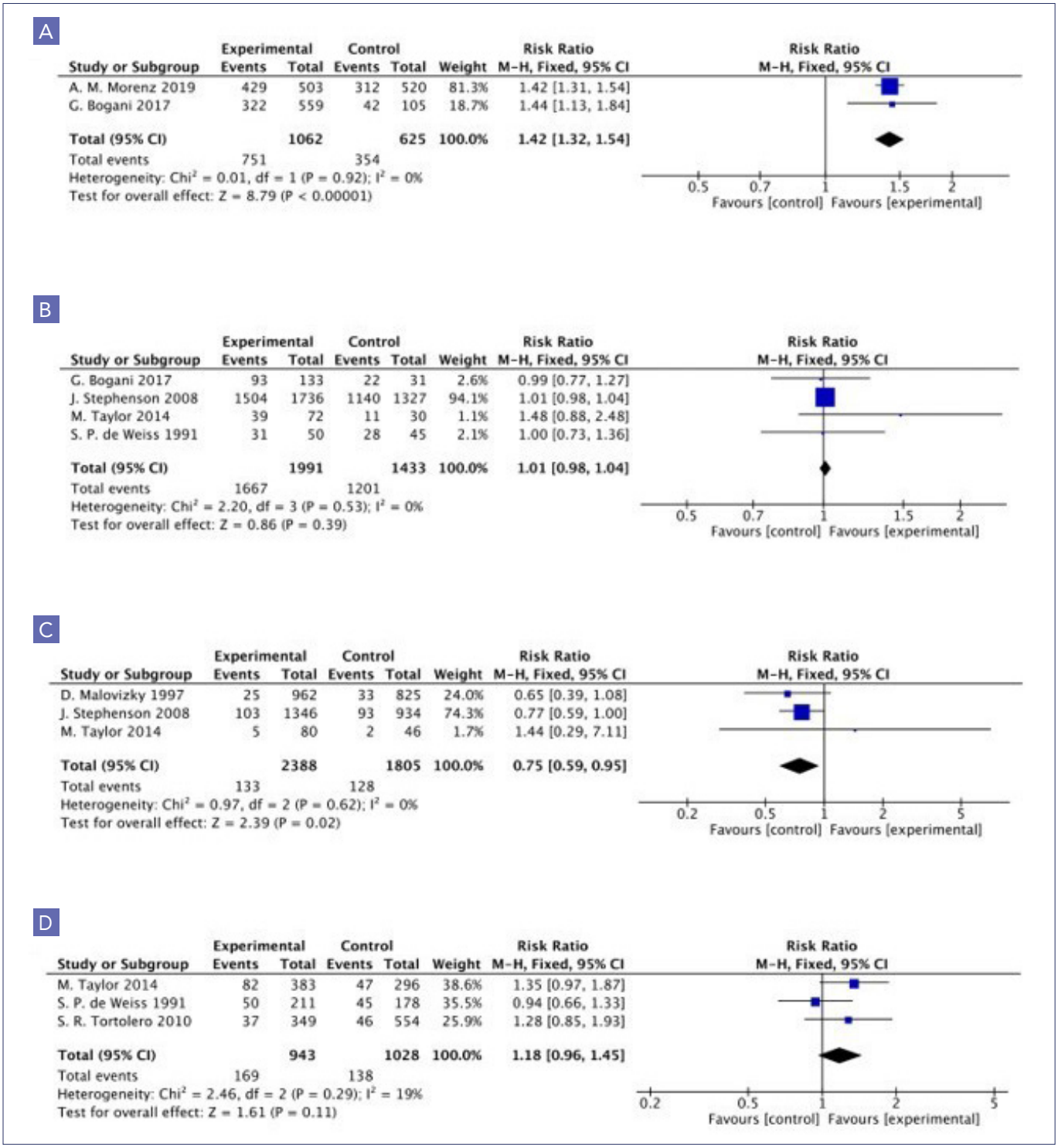

Figure 3. *Meta-analysis: (**A**) of knowledge of contraceptive methods; (**B**) of the use of contraceptives before/after sexual education programs; (**C**) of the incidence of unwanted pregnancy; (**D**) of sexual experience in two groups.*

sexual experience (66.0 (61.6-70.2)), whereas a no-education group had the least (11.5 (8.9-14.7)), and an abstinence-only sex education group's results were in the middle (22.6 (19.1-26.5) ($p < 0.06$)). In de Weiss *et al*.'s study [32] adolescents without sex education had bigger sexual experience; however in Taylor *et al*.'s and Tortolero *et al*.'s studies [30, 34], conversely, sex education group demonstrated a bigger sexual experience than adolescents who didn't receive sex education.

The fourth analysis was done also as a meta-analysis of three studies [30, 32, 34]: RR = 1.18, 95%CI 0.96 to 1.45, p = 0,11. The heterogeneity for this comparison was 19%. Our analysis showed that there is

no significant difference in the sexual experience between adolescents who received sex education compared to those who did not receive it (**Figure 2**).

Knowledge of HIV/STD transmission

In Bogani *et al*.'s study [26] knowledge about the transmission of HIV and STDs among adolescents was assessed, and the results showed that students who received sexual education courses had better knowledge than those who didn't (82% *vs* 71% (p = 0.01)). The same data was demonstrated by the Tortolero *et al*. study [30]. Also, in Tortolero *et al*.'s study 26 post-educational results of adolescents were much better than before sex education among both boys and girls ($p < 0.001$).

STD diagnosis

In Kohler *et al*.'s study [27] group of adolescents who received comprehensive sex education demonstrated the highest rate of STD (73.4 (59.5-83.8)). In the second place was the group that received abstinence-only sex education (19.7 (11.1-32.6)), and no education group was in last place (6.9 (3.0-15.4) (p = 0.55)).

Knowledge of pregnancy

A study by Morenz *et al*.'s [29] demonstrated that the sex education lesson had significantly increased the level of knowledge about pregnancy (36% for the pre-test *vs* 92% for the post-test group).

Abortion rate

Stephenson *et al*.'s study [33] assessed abortion rates among adolescents, demonstrating that it was higher in the group that attended teacher-led sex education compared with the group that attended peer-led sex education, including adolescents (5.11% *vs* 3.91%).

DISCUSSION

Sexual education plays a vital role in shaping the sexual health outcomes of adolescents worldwide. At the same time, it has always been a sensitive topic and causes a lot of controversy.

This study aimed to evaluate the impact of sexual education on the sexual knowledge of adolescents, unwanted pregnancy, abortions, and sexually transmitted diseases, pregnancy rate, sexual initiation, incidence of STDs, contraceptive use, abortion rates, and knowledge of STDs, pregnancy, and contraceptive methods. The findings of our systematic review and meta-analysis highlight the statistically significant importance of implementing sex education regarding the following topics: knowledge about contraception methods (RR = 1.42, 95%CI 1.32 to 1.54, $p < 0.00001$) and rate of unwanted pregnancies (RR = 0.75, 95%CI 0.59 to 0.95, p = 0.02). However, the data obtained on the impact of sexual education on the level of contraceptive use (RR = 1.01, 95%CI 0.98 to 1.04, p = 0.39) and sexual experience of adolescents (RR = 1.18, 95%CI 0.96 to 1.45, p = 0.11) were not reliable and need further investigation. Particular attention should be paid to the study of the impact of sex education on the use of contraceptives by adolescents, which directly affects the rate of unplanned pregnancies and unsafe abortions, which in turn causes significant harm to the health of adolescents and entails economic and social difficulties [35].

Results of our work correlate with the results of the following systematic review by Gamelia *et al*. [36] in terms of potentially significant decreasing of teenage pregnancy, STDs, and risky sexual behaviour. Furthermore, scientific evidence collected by the World Health Organization together with UNESCO and several other international organizations proves that introducing sexuality education in schools would be beneficial to society [37].

Sex education promotes good attitudes regarding sexual and reproductive health – and thus reduces the risks of contracting HIV and sexually transmitted infections. For example, in countries where sex education is organized at a systemic level – such as the Netherlands, Finland, and Sweden - the incidence rates of HIV/STIs and early pregnancy and, as a result, abortions are much lower [37, 38].

Every year, an estimated 21 million girls aged 15-19 years in developing regions become pregnant, and approximately 12 million of them give birth [38]. Sex education classes should reduce the number of abortions among teenagers. In many countries, adolescents are unable to avoid unwanted pregnancy due to circumstances that prevent them from obtaining and using contraceptives [37].

Our systematic review and meta-analysis includes 9 studies out of 1,512 publications, and this is the first study that assessed the effectiveness of adolescent sex education in terms of pregnancy rate, sexual initiation, incidence of STDs, contraceptive use, abortion rates, and knowledge of STDs, pregnancy, and contraceptive methods.

Moreover, during the research we have found a great number of studies that demonstrated the po-

sitive impact of sexual education in adolescents on various aspects that we did not analyse, such as knowledge about sexual health and sexual health services, attitudes about sexual relationship rights, communication about sex and relationships with parents, and self-efficacy to manage risky situations at immediate post-test [39].

Thus, sexual education is a complex subject; it covers a wide range of topics, for instance, bullying, depression, anxiety, low self-esteem, substance abuse, suicide attempts, and unstable housing, which are being discussed in several studies [40]. These questions were not the focus of our study but needed to be reviewed in the future.

Limitations of our systematic review and meta-analysis were the small number of included studies and participants that made it difficult to generalize the results. In addition, some of the studies included in this article were of low quality and presented moderate and critical risk of bias, which also negatively affected the results of data analysis. We need more high-quality clinical trials and low risks of bias to discuss this problem more evidence-based. Some studies included not only female patients but also male patients. Another limitation of the studies is relatively short follow-up and subsequent inability to assess the long-term prospects for unwanted pregnancies. One more limitation of our study is the high heterogeneity of the included groups of participants, which was influenced by various school sexual education programs, as well as cultural distortions, which did not allow for a qualitative analysis of the data and negatively affected the results.

Concerning implications for future research, they may include well-performed study design and long-term follow-up to assess the impact of sexual education on adolescents. Also, more studies and meta-analysis evaluating the impact of sex education on abortion rates in adolescence are needed. It is also necessary to divide studies on the impact of sex education by gender. And decide at what age sex education lessons are most effective.

Overall, this systematic review and meta-analysis highlights the positive impact of comprehensive sex education on the sexual health outcomes of adolescents and underscores the importance of continued research in this area. By prioritizing evidence-based approaches and promoting a holistic understanding of sexuality, we can empower young people to make informed choices and lead healthy, fulfilling lives.

CONCLUSIONS

The present systematic review and meta-analysis suggested that school-based sexual education programs improve, in a statistically significant way, sexual knowledge among adolescents regarding the following topics: knowledge about contraception methods and rate of unwanted pregnancies, but the difference in the level of contraceptive use and sexual experience of adolescents was not statistically significant. Adults have to overcome taboo and reluctance to confront sexual issues with adolescents. Comprehensive sexual and reproductive health education and counselling could potentially avert a significant number of teenage pregnancies in these settings. Further research is needed in order to form a more complete understanding of the role of sexual education in the sexual health of adolescents.

COMPLIANCE WITH ETHICAL STANDARDS

Authors' contribution

L.P., J.A.: Conceptualization, methodology, project administration, supervision, formal analysis. E.K., Y.D.: Writing – review & editing, validation, visualization. K.K., E.Z.: Investigation, writing – original draft.

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Study registration

PROSPERO international prospective register of systematic reviews by the National Institute for Health Research (NIHR). The registration number is PROSPERO 2022 CRD42022357877.

Disclosure of interests

The authors declare that they have no conflict of interests.

Ethical approval

N/A.

Informed consent

N/A.

Data sharing

Data are available along with the review.

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Evaluating the role of endometrial thickness on hCG injection day: a predictive marker for reproductive success in clomiphene-IUI cycles

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ABSTRACT

Objective. Endometrial thickness (EMT) on the day of hCG administration is critical for predicting reproductive success in intrauterine insemination (IUI) cycles with clomiphene citrate (CC). However, the optimal EMT for clinical pregnancy and live birth outcomes remains unclear. In this study, the influence of EMT on reproductive outcomes in CC+IUI cycles is investigated.

Materials and Methods. This retrospective analysis included 640 IUI cycles performed at a tertiary reproductive endocrinology centre between February 2019 and February 2020. Participants were couples with unexplained infertility or WHO category 2 anovulation. All underwent ovulation induction with CC, and EMT was measured via transvaginal ultrasound on the day of the hCG trigger. Outcomes included clinical pregnancy and live birth rate.

Results. Of 640 cycles, 80 (12.5%) achieved clinical pregnancy, with 52 (8.1%) resulting in live births. Spontaneous abortion occurred in 23 cases (3.6%). Age, duration of infertility and CC dose had a significant impact on clinical pregnancy and live birth rates. The optimal EMT threshold for predicting clinical pregnancy was 8.45 mm (sensitivity 49.4%, specificity 55.3%). An EMT between 8-9 mm was associated with higher clinical pregnancy ($p = 0.010$) and live birth rates ($p = 0.002$).

Conclusions. EMT has a significant impact on pregnancy outcome in CC+IUI cycles, with 8-9 mm being the optimal range for better outcomes. However, EMT alone shows moderate predictive accuracy and should be combined with other clinical factors in decision making. Further research is needed to refine prediction models that integrate EMT, patient age and duration of infertility to improve outcomes.

INTRODUCTION

Infertility affects an estimated 8-12% of couples of reproductive age and has an increasing trend. It is estimated that 1 in 7 couples in Western countries is affected by infertility, compared to 1 in 4 couples in developing countries [1-4]. Unexplained infertility (UI) occurs in 15% of all cases of infertility. In

women, UI is associated with older age, lower BMI, lower endometrial thickness, and poorer ovarian reservation testing [1-4]. The World Health Organization (WHO) task force on Diagnosis and Treatment of Infertility performed a study on 8,500 infertile couples utilizing standard diagnostic criteria and reported female factor infertility in 37%, male factor infertility in 8% of the couples while both male and female factor infertility was present in 35% of the couples in developed countries. Meanwhile, 5% of the couples had unexplained infertility and 15% became pregnant during the study [5].

Clomiphene citrate (CC), a selective estrogen receptor modulator (SERM), has been widely used for 40 years as it is the first agent used for ovulation induction. CC is used for ovulation induction in combination with intrauterine insemination (IUI) in normogonadotropic anovulatory women and women with unexplained infertility. When endogenous estrogen levels are sufficient, CC acts as a competitive estrogen antagonist, whereas at low endogenous estrogen levels it exhibits estrogenic properties. CC binds to the nuclear estrogen receptors (ER) in the subthalamic area and thus depletes hypothalamic ERs by blocking the negative feedback effect of circulating endogenous estradiol (E2) [6]. This leads to stimulation of the pulse rate of hypothalamic gonadotropin-releasing hormone (GnRH), resulting in increased serum concentrations of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) [7]. The elevated FSH and LH concentrations stimulate follicular development in the ovaries. CC acts primarily as an anti-estrogen on the uterus, cervix and vagina, so the expected normal increase in uterine volume and endometrial thickness (EMT) that occurs in spontaneous menstrual cycles is largely absent during clomiphene-induced cycles, although E2 levels are high [8]. The use of clomiphene citrate (CC) is associated with the development of a thinner endometrium in 15% to 50% of patients [9]. The thinner endometrium is one of the factors blamed for the discrepancy between the ovulation rate and the pregnancy rate in CC-induced cycles [10].

The role of the EMT and normal trilaminar appearance evaluated during ultrasonographic examination have been extensively studied in *in vitro* fertilization-embryo transfer (IVF-ET) cycles as these parameters have been proposed to be predictors of endometrial receptivity and thus reproductive outcome [11].

In the presented study we aimed to evaluate the effect of EMT on the day of Human Chorionic Gonadotropin (hCG) administration on the reproductive outcomes and determine the threshold value for EMT for achieving clinical pregnancy in CC + IUI cycles.

MATERIALS AND METHODS

Study design

The results of the 640 cycles in couples who received CC+IUI treatment at Reproductive Endocrinology Department of Health Sciences University Etlik Zübeyde Hanım Women's Training and Research Hospital between February 2019 and February 2020 for unexplained infertility or WHO Category 2 anovulation (normogonadotropic anovulatory women) and met the inclusion criteria were analysed retrospectively. Our study was approved by the Ministry of Health, Health Sciences University Etlik Zübeyde Hanım Women's Institutional Board (30/12/2020-90057706-799).

Day-3 FSH (Elecsys FSH, Roche), LH (Elecsys LH, Roche), Prolactin (Elecsys Prolactin II, Roche), thyroid-stimulating hormone (TSH) (Elecsys TSH, Roche) levels and Day-21 progesterone (Elecsys Progesterone, Roche) levels are analysed. Basal transvaginal ultrasonography (TVUSG), basal antral follicle count (AFC), and hysterosalpingography (HSG) were performed as a part of the routine infertility workup. Two sperm samples of the partners were examined after urological examination, and the presence of male factor was evaluated according to the WHO criteria [12].

Inclusion-Exclusion criteria

During the assessment phase, the causes of infertility were carefully evaluated for each patient. This evaluation included a thorough review of medical histories, physical examinations, and diagnostic tests to identify any underlying factors contributing to infertility. Based on these findings, any necessary adjustments were made to the treatment protocol, including modifications to the doses of medications used or alterations in the duration of follicular growth stimulation. These adjustments were personalized to optimize the chances of a successful outcome. Women with under 40 age, ovulatory dysfunction (WHO Category 2) or unexplained infertility (ovulatory women with at least one patent tube on HSG and follicular growth on the

side of the patent tube and absence of male infertility) were included in the study. Women with the duration of infertility less than a year, bilateral tubal occlusion, a basal FSH ≥ 15 mIU/ml, basal estradiol ≥ 80 pg/ml or ≤ 20 pg/ml, additional endocrine disease (such as Cushing, diabetes, thyroid dysfunction, hyperprolactinemia, androgen-synthesizing tumour, 21-hydroxylase enzyme defect) and who failed to demonstrate follicular growth during the CC treatment cycle or did not receive intrauterine insemination were excluded from the study.

Treatment protocol

CC (Klomen®; Kocak Farma / Turkey, Serophene®; Merck Serono / Italy) was commenced on Day-3 and was continued for five days after the onset of spontaneous menses after a TVUSG evaluation. A standard dose of 50 mg/day was used for the first treatment cycle, but the treatment doses were increased by 50 mg increments if the patients failed to achieve follicular growth with the given dose during the previous treatment cycle. The maximum CC dose was 150 mg/day according to our protocol. The women were scheduled for IUI 36 hours after subcutaneous hCG injection (250 μg recombinant hCG Ovitrelle®; Merck-Serono / Italy) when a follicle reached to a diameter > 17mm. hCG was used to trigger ovulation because it allows for the optimization of timing in terms of follicle development and endometrial preparation. The main reason for using hCG instead of monitoring natural ovulation is that the timing of the LH surge and natural ovulation is unpredictable, which can make IUI timing difficult. Triggering with hCG allows for the best timing of sperm to egg, allowing for controlled ovulation. Additionally, the use of hCG is more consistent compared to the LH surge observed in natural cycles, which can improve pregnancy rates. The patients who had more than 2 follicles with a diameter >17 mm were not scheduled for intrauterine insemination in order to avoid multiple pregnancies. Estradiol levels were measured in all participants before hCG injection. The mean estradiol level was determined as 362 pg/ml. The estradiol threshold determined for hCG triggering was accepted as 1,500 pg/ml; hCG was not administered to induce ovulation in patients below this threshold. This threshold value, together with endometrial thickness and follicle development, was used to determine the optimal timing. EMT was measured on the day of hCG injection in the midsagittal plane of the uterus as the maximum distance between the two inner faces of the endometrial myometrial junction and recorded.

Semen samples were collected by masturbation following 3-5 days of sexual abstinence. The seminal parameters were analysed by a single observer and categorized according to the 2021 WHO criteria [12]: complete liquefaction within 60 minutes; sperm concentration ≥ 15 million per ml; progressive motility (PR) ≥ 32%; total motility (progressive + non-progressive, PR + NP) ≥ 40%; and normal morphology ≥ 4%. The ejaculate was prepared using a swim-up technique. IUI was performed by injecting the prepared semen into the intrauterine cavity using a soft insemination catheter (AINSEBLUE-R RI. MOS. ITALY). For luteal phase support, a standardized protocol was employed, which involved the administration of vaginal 200 mg progesterone twice a day. This was initiated following the insemination procedure and continued throughout the luteal phase to support the endometrium and enhance the likelihood of implantation. Success criteria after IUI were taken as clinical pregnancy (CP) and live birth (LB). CP was diagnosed with a positive β-hCG (> 10 mIU/ml) on the 14th day after IUI followed by observing an intrauterine gestational sac during TVUSG in the 5th week as described by Zhang *et al*. [13]. LB was defined as the delivery of a viable infant at 24 weeks gestation or later. The absence of a gestational sac on TVUSG despite an elevated β-hCG was defined as biochemical pregnancy [14]. The findings were compared by comparing the variables of the group with CP with the ones without CP.

Statistical analysis

SPSS 21.0 package program was used to analyse the data. Numerical data are mean ± standard deviation, data on categorical variables number (percentage). The compliance of numerical variables to normal distribution was checked by the Shapiro Wilk test. The numerical data conforming to the normal distribution assumption were compared with the independent sample t-test, the numerical data not compatible with the normal distribution assumption with the Mann-Whitney U test, and the categorical variables with the Pearson's Chi-square test. Statistical significance was accepted as $p < 0.05$. The sample size was determined based on the statistical power analysis of previous similar study [15]. The minimum sample size required to detect a significant difference with 90% power and 5% margin of error was calculated as 600. However,

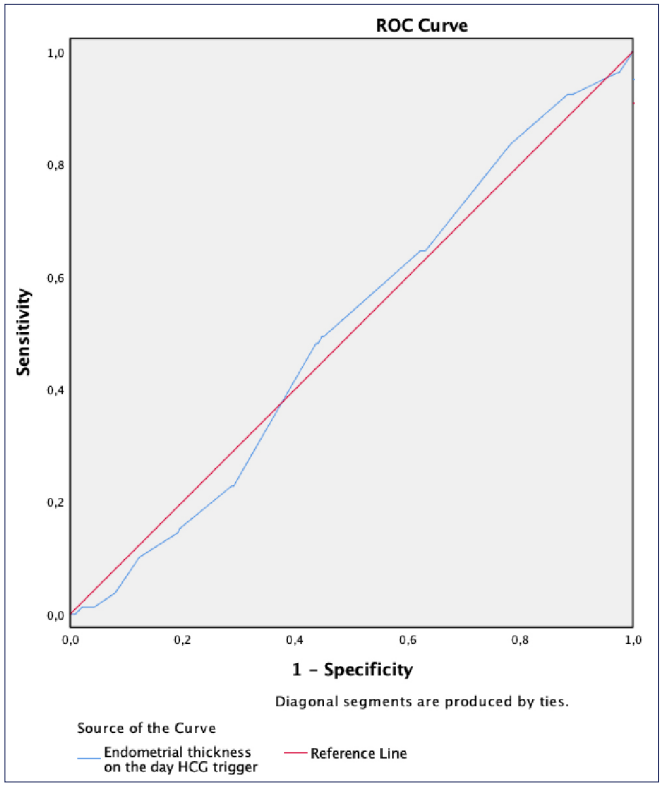

Figure 1. *Receiver operator characteristic (ROC) curve of endometrial thickness on the day of hCG trigger.*

The ROC curve illustrates the diagnostic ability of endometrial thickness (EMT) on the day of hCG trigger for predicting clinical pregnancy in CC+IUI cycles. The optimal cut-off value for EMT was determined to be 8.45 mm, with a sensitivity of 49.4% and specificity of 55.3%. Despite its moderate predictive accuracy, the results suggest that EMT can serve as a useful biomarker for assessing uterine receptivity, although it should not be used as a sole criterion for cycle continuation or cancellation. The analysis emphasizes the importance of considering other factors, such as female age and duration of infertility, in conjunction with EMT for clinical decision-making.

Table 1. *The demographic characteristics and clinical features of the patient group compared according to the presence of live birth.*

	Live Birth		P-value
	None (n = 588)	Yes (n = 52)	
Age	28.01 ± 4.95	26.31 ± 5.02	0.018*
Infertility type			
Primary	407 (69.22%)	35 (67.31%)	0.775
Secondary	181 (30.78%)	17 (32.69%)	
Duration of marriage	4.37 ± 3.28	4.17 ± 3.82	0.682
Duration of infertility	3 ± 1.94	2.52 ± 2.06	0.086
AMH (ng/ml)	4.45 ± 3.09	4.83 ± 3.39	0.436
FSH (mIU/ml)†	7.04 ± 2.06	6.94 ± 1.71	0.740
LH (mIU/ml)†	5.8 ± 4.46	5.49 ± 3.04	0.628
E2 (pg/ml)†	48.7 ± 20.13	46.1 ± 14.03	0.125
PRL (mg/dl)†	14.19 ± 7.96	13.08 ± 7.32	0.348
TSH (µg/dl)†	1.99 ± 1.22	2.14 ± 1.16	0.375
Body mass index (kg/m^2)	24.8 ± 4.33	25.02 ± 5.32	0.734
Basal USG EMT (mm)	4.61 ± 1.79	4.3 ± 1.68	0.236
Daily CC dose (mg)	57.48 ± 20.31	62.5 ± 25.96	0.180
Ovarian Response			
Duration of follicular growth (day)	11.11 ± 2.81	12.04 ± 2.92	0.024*
Number of Follicle	1.23 ± 0.52	1.1 ± 0.36	0.014 *
Diameter of the dominant follicle (mm)	19.34 ± 1.85	19 ± 1.36	0.203
EMT on the day HCG trigger (mm)	8.47 ± 2.55	8.38 ± 2.11	0.802

EMT: Endometrial Thickness; TSH: Thyroid-stimulating hormone; FSH: Follicle-stimulating hormone; LH: Luteinizing hormone; E2: Estradiol; PRL: Prolactin; AMH: Anti-mullerian hormone; CC: Clomiphene citrate; USG: Ultrasonography; *P-value < 0.05 statistically significant; †Measurement on the day of the early follicular phase.

a total of 640 cycles were analysed throughout the study, which increases the statistical power of our study and reinforces the reliability of the findings.

RESULTS

Out of the 640 cycles evaluated, elevated β-hCG (> 10 mIU/ml) was observed in 92 (14.4%). Twelve (1.8%) of these cases were biochemical pregnancies as the β-hCG level declined and a gestational sac was not observed while 80 of the cases (12.5%) achieved clinical pregnancy. Ectopic pregnancy was not observed in the study group. While 23 (23/640, 3.6%) of the cases with CP had spontaneous abortion (SA), 52 (52/640, 8.1%) resulted in live birth (LB) (**Table 1**). 5 cases were not followed-up till the end of pregnancy. The patients with CP were significantly younger (p = 0.002), the duration of infertility was significantly shorter (p = 0.002), and the CC dosage was statistically significantly higher (p = 0.034) when compared to the group who failed to achieve a CP (**Table 2**). The age was statistically lower in patients who had an LB (p = 0.018).

We compared biochemical pregnancy, CP, SA, and LB. The incremental EMT measurement results were starting to ≤ 6 up to > 15 mm. The rate of clinical pregnancy (CPR), live birth (LBR), and spontaneous abortion (SAR) were evaluated in relation to the endometrial thickness (**Table 3**).

The mean EMT was found to be 8.38 mm in cases with a CP, and the cut-off point for EMT on the day hCG trigger was 8.45 (sensitivity of 49.4.% and specificity of 55.3%) for CP (**Figure 1**). When we compare the EMT the incidence of biochemical pregnancy was lower (p = 0.013), and CPR (p = 0.010) and LBR (p = 0.002) were higher in cases with an EMT was between 8 -9 mm on the day of β-hCG injection. There was no significant difference in the EMT in terms of the incidence of SA (p = 0.579) (**Table 3**). The distribution of statistically significant data was given in **Table 4**. A multivariate

Table 2. *The demographic characteristics and clinical features of the patient group compared according to the presence of clinical pregnancy.*

	Clinical Pregnancy		P-value
	None (n = 560)	Yes (n = 80)	
Age	28.1 ± 4.99	26.29 ± 4.62	0.002*
Infertility type			
Primary	387 (69.11%)	55 (68.75%)	0.948
Secondary	173 (30.89%)	25 (31.25%)	
Duration of marriage	4.41 ± 3.3	3.95 ± 3.49	0.245
Duration of infertility	3.04 ± 1.96	2.44 ± 1.81	0.011*
AMH (ng/ml)	4.44 ± 3.1	4.79 ± 3.16	0.368
FSH (mIU/ml)†	7.07 ± 2.06	6.77 ± 1.83	0.218
LH (mIU/ml)†	5.71 ± 3.8	6.25 ± 7.19	0.303
E2 (pg/ml)†	48.3 ± 20.19	46.6 ± 17.01	0.495
PRL (mg/dl)†	14.09 ± 7.9	14.15 ± 7.97	0.955
TSH (µg/dl)†	1.98 ± 1.2	2.15 ± 1.26	0.252
Body mass index (kg/m^2)	24.82 ± 4.33	24.77 ± 5.02	0.920
Basal USG EMT (mm)	4.61 ± 1.81	4.38 ± 1.61	0.283
Daily CC dose (mg)	57.23 ± 20.21	62.5 ± 24.52	0.034*
Ovarian Response			
Duration of follicular growth (day)	11.11 ± 2.82	11.75 ± 2.88	0.058
Number of Follicle	1.24 ± 0.53	1.11 ± 0.36	0.007*
Diameter of the dominant follicle (mm)	19.34 ± 1.84	19.1 ± 1.68	0.275
EMT on the day HCG trigger (mm)	8.47 ± 2.56	8.38 ± 2.16	0.766

EMT: Endometrial Thickness; TSH: Thyroid-stimulating hormone; FSH: Follicle-stimulating hormone; LH: Luteinizing hormone; E2: Estradiol; PRL: Prolactin; AMH: Anti-mullerian hormone; CC: Clomiphene citrate; USG: Ultrasonography. *P-value < 0.05 statistically significant; †Measurement on the day of the early follicular phase.

logistic regression analysis was performed to evaluate the independent effects of various variables on live birth rates. The results showed that age was a statistically significant predictor (OR 0.91, 95%CI 0.86-0.98, p = 0.019), with increasing age negatively affecting the likelihood of live birth. However, endometrial thickness (EMT) did not demonstrate independent predictive significance for live birth outcomes (OR 0.99, 95%CI 0.88-1.11, p = 0.86). Similarly, the duration of follicular growth and the number of dominant follicles were not significant predictors of live birth rates in this model (**Table 5**).

Our results demonstrate that an optimal EMT of 8-9 mm is associated with significantly higher clinical pregnancy and live birth rates, suggesting that EMT could be a useful biomarker for assessing uterine receptivity in CC+IUI cycles, despite its moderate predictive accuracy (sensitivity of 49.4% and specificity of 55.3%) (**Figure 1**).

DISCUSSION

This study aimed to evaluate the relationship between endometrial thickness (EMT) on the day of hCG injection and reproductive outcomes in patients undergoing ovulation induction with clomiphene citrate (CC) combined with intrauterine insemination (IUI). Our findings indicate that an

Table 3. *Endometrial Thickness (EMT) on the day of hCG administration.*

EMT (mm)	Number of patients (n = 640)	Biochemical Pregnancy (n = 12)	Clinical Pregnancy (n = 80)	Spontaneous Abortion (n = 23)	Live Birth (n = 52)
≤6	135	3 (2.22%)	13 (9.63%)	4 (2.96%)	8 (5.93%)
>6 - ≤7	100	4 (4%)	15 (15%)	5 (5%)	9 (9%)
>7 - ≤8	113	1 (0.88%)	12 (10.62%)	5 (4.42%)	7 (6.19%)
>8 - ≤9	111	0 (0%)	22 (19.82%)	3 (2.7%)	17 (15.32%)
>9 - ≤10	60	2 (3.33%)	6 (10%)	2 (3.33%)	4 (6.67%)
>10 - ≤11	44	0 (0%)	4 (9.09%)	1 (2.27%)	3 (6.82%)
>11 - ≤12	2	1 (3.45%)	5 (17.24%)	2 (6.9%)	3 (10.34%)
>12 - ≤13	23	0 (0%)	2 (8.7%)	1 (4.35%)	0 (0%)
>13 - ≤14	12	0 (0%)	0 (0%)	0 (0%)	0 (0%)
>14 - ≤15	8	0 (0%)	1 (12.5%)	0 (0%)	1 (12.5%)
>15	5	1 (20%)	0 (0%)	0 (0%)	0 (0%)
Distribution of results by endometrial thickness (> 8 & 9) on the day of HCG					
8 - 9 mm	111	0 (0%)	22 (19.82%)	3 (2.7%)	17 (15.32%)
<8 - >9 mm	539	12 (2.27%	58 (10.96%)	20 (3.78%)	35 (6.62%)
p	N/A	0.013*	0.010*	0.579	0.002*

EMT: Endometrial Thickness; HCG: Human Chorionic Gonadotropin. Data are shown as numbers (percentages). *Statistically significant; P-value < 0.05 statistically significant.

Table 4. *Distribution of statistically significant data.*

	Age	Duration of infertility	Daily clomiphene citrate dose (mg)	Number of Follicle	Duration of Follicular growth
Clinical Pregnancy (+) (n = 80)	26.29	2.44	62.5	1.11	11.75
Clinical pregnancy (-) (n = 560)	28.1	3.04	57.23	1.24	11.11
P-value	**0.02***	**0.011***	**0.034***	**0.007***	**0.058**
Live Birth (+) (n = 52)	26.31	2.52	62.5	1.1	12.04
Live Birth (-) (n = 588)	28.01	3	57.48	1.23	11.11
P-value	**0.018***	**0.086**	**0.180**	**0.014***	**0.024***
Spontaneous abortion (+) (n = 23)	26.57	2.39	65.22	1.09	10.96
Spontaneous abortion (-) (n = 57)	26.31	2.52	62.50	1.10	12.04
P-value	**0.704**	**0.631**	**0.001***	**0.903**	**0.138**

*Statistically significant; P-value < 0.05 statistically significant.

Table 5. *Variables associated with live birth analysed by multivariate logistic regression.*

		Standardized Coefficients		
Variable	**Beta**	**Standardized Error**	**Odds ratio (CI)**	**P-value**
Age	-0.95	0.002	0.91 (0.86-0.98)	0.019*
Duration of Follicular Growth	0.74	0.007	0.51 (0.22-1.17)	0.72
Number of Dominant Follicile	-0.53	0.021	0.93 (0.81-1.07)	0.186
Endometrial Thickness	-0.01	0.59	0.99 (0.88-1.11)	0.86

OR: Odds ratio; CI: Confidence interval; P-value < 0.05 statistically significant; *statistically significant.

EMT of 8-9 mm is associated with significantly higher clinical pregnancy and live birth rates, suggesting that EMT could serve as a useful biomarker for uterine receptivity in CC+IUI cycles, albeit with moderate predictive accuracy.

Several studies have explored the predictive value of EMT in assisted reproductive techniques. Our results align with previous research indicating that certain EMT thresholds, particularly between 8 and 9 mm, are associated with higher pregnancy success rates [16, 17]. This threshold is consistent with the findings of Huniadi *et al*. [18], who also reported that EMT, along with other factors such as the duration of infertility and patient age, significantly predicts the success of IUI procedures. They emphasized that integrating these factors into a prognostic model could enhance patient counselling and improve treatment outcomes [18].

Our findings also reveal that EMT alone may not be sufficient to predict IUI outcomes reliably, as its sensitivity and specificity were moderate. This is consistent with studies by. Masrour *et al*. and Kolibianakis *et al*, which found no significant difference in EMT between women who achieved pregnancy and those who did not [15,19]. These results suggest that while EMT is a useful indicator, it should be evaluated alongside other factors such as age, hormonal levels, and ovarian response to provide a more comprehensive prediction model [20].

Additionally, the multivariate analysis revealed that age was the only variable significantly associated with live birth rates, with a negative impact observed as age increased. This finding aligns with previous studies that highlight the detrimental effects of advanced maternal age on reproductive outcomes due to factors such as reduced oocyte quality and increased aneuploidy rates [21, 22]. Contrary to expectations, EMT did not independently predict live birth in our model. This may reflect the moderate predictive accuracy of EMT observed in univariate analyses, emphasizing its limited standalone utility. Furthermore, neither the duration of follicular growth nor the number of dominant follicles showed significant associations with live birth outcomes, suggesting that these factors may play a secondary role compared to age [23, 24]. Besides, our study highlighted that shorter infertility duration was associated with higher clinical pregnancy rates, which aligns with other studies reporting similar findings [25, 26]. However, there is no consensus on the ideal duration of infertility for achieving favourable outcomes during treatment cycles.

In conclusion, while EMT remains a valuable biomarker for assessing uterine receptivity in CC+IUI cycles, it should not be the sole criterion for clinical decisions. The moderate predictive accuracy of EMT underscores its limitations as a standalone biomarker. While our findings suggest that an EMT of 8-9 mm improves reproductive outcomes, its clinical utility must be contextualized within a broader framework of patient-specific factors. These findings highlight the need for multifactorial prediction models to improve decision-making in clinical practice. A multifactorial approach that includes patient age, infertility duration, ovarian response, and specific infertility conditions should be adopted to enhance the predictive accuracy of IUI outcomes and optimize treatment strategies. Future research should focus on developing and validating comprehensive predictive models that incorporate these variables to better guide clinical practice.

Strengths and limitations

To the best of our knowledges, this study is one of the largest retrospective study which is searching only CC-IUI from the assisted reproductive technique. In addition, one of the strongest aspects of this study is that it is one of the few studies that tried to find a threshold value for EMT on the day of the hCG trigger in infertile couples who underwent CC-IUI, and also gave concretely viable pregnancy and spontaneous abortion rates according to the threshold value. Inclusion of only those with unexplained infertility and WHO category 2 normogonodotropic anovulatory patients in the study resulted in a more homogeneous patient group.

There are also many limitations. One of these limitations is the retrospective design. The retrospective nature of this study limits its ability to establish causality. Biases in data collection, including incomplete medical records and variability in ultrasound measurements, may have influenced the findings. Furthermore, the homogeneity of the study population, while providing consistency, limits the generalizability of the results to more diverse populations. Apart from this, although this study has a sufficient sample size to give an EMT threshold value, the low sensitivity and specificity of the threshold value obtained indicate that prospective studies with larger sample sizes are needed to give a threshold value with higher sensitivity and specificity. In addition, ultrasound measurements are inherently subjective even though the technique is standardized across the reproductive endocrinology clinics as mentioned in the method section. Among the possible limitations of the present study, endometrial receptivity is not only influenced by EMT, but also by factors such as endometrial pattern and vascularity, which were not evaluated in this study. The absence of these parameters is a limitation, as their inclusion would allow a more comprehensive assessment of uterine receptivity. Another limitation of this study was that endometrial thickness was not measured in 2-3 day cycles. Perhaps midcycle endometrial thickness is related to early follicular phase thickness.

Given these discrepancies, further prospective, large-scale studies are necessary to validate the role of EMT as a reliable predictor of reproductive outcomes in IUI cycles. Such research should aim to control for the various confounding factors that may influence EMT, including patient demographics, treatment protocols, and ultrasound measurement techniques. Moreover, exploring the interaction between EMT and other factors, such as ovarian response and hormonal profiles, could help develop a more comprehensive predictive model for IUI success.

CONCLUSIONS

In conclusion, our study found that an endometrial thickness (EMT) of 8-9 mm on the day of hCG trigger is associated with higher clinical pregnancy and live birth rates in CC+IUI cycles. However, due to the low sensitivity and specificity, and successful pregnancies occurring outside this range, setting a strict EMT threshold for cycle decisions is not advisable. Other factors such as female age and duration of infertility should also be considered. These findings can help optimize patient selection and treatment regimens, reducing unnecessary interventions and costs.

COMPLIANCE WITH ETHICAL STANDARDS

Authors' contribution

A.A.: Conceptualization. A.A., G.A.E., D.S.: Data curation, formal analysis. A.A., B.D., A.G.E., S.D..: Investigation, project administration, visualization. A.A., B.D.: Methodology. B.D, S.D.: Supervision, validation. A.A.: Writing – original draft. A.A., B.D., S.D.: Writing – review & editing.

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Informed consent
As this study was retrospective and all data were anonymized prior to analysis, the Institutional Review Board waived the requirement for informed consent.

Data sharing
Data are available under reasonable request to the corresponding author.

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A diagnostic challenge: spontaneous hemoperitoneum in pregnancy *versus* uterine rupture

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ABSTRACT

Background. Spontaneous hemoperitoneum in pregnancy (SHiP) is a rare but life-threatening complication. SHiP typically presents with abdominal pain, hypovolemia, and decreased haemoglobin in later pregnancy.

Case presentation. A 38-year-old woman with a history of one prior caesarean section, and otherwise in good health, presented at 29 weeks of gestation with sudden onset of abdominal pain. Ultrasound revealed free fluid in her abdomen, raising concern for uterine rupture due to the previous caesarean section. However, a dedicated ultrasound examination ruled this out. Laparoscopy confirmed the absence of uterine involvement. The lower uterine segment was intact and was not the source of bleeding; indeed, the source of bleeding was identified as a 2-cm clot in the left fallopian tube resulting on histology an endometriosis foci.

Conclusions. This case highlights the importance of considering SHiP in the differential diagnosis of acute abdomen during pregnancy, even in the absence of known risk factors because a high index of suspicion for SHiP is crucial for prompt diagnosis and intervention, aiming for optimal maternal and feta outcomes.

BACKGROUND

The differential diagnosis of pain and sonographic findings of intra-abdominal effusion during pregnancy or in the hours immediately following birth is complex and requires careful consideration of various potential causes. Uterine rupture [1], a life-threatening obstetric emergency, must be promptly excluded. However, other conditions such as spontaneous hemoperitoneum in pregnancy (SHIP), bladder rupture [2], and ascites [3] can present with similar clinical features. A thorough evaluation, including imaging studies, laboratory tests, and clinical assessment, is essential to differentiate between these conditions and ensure appropriate management.

In particular, spontaneous hemoperitoneum in pregnancy (SHiP) is defined as a sudden non-traumatic intraperitoneal bleeding in pregnancy and up to 42 days postpartum [4]. SHiP typically presents during the latter stages of pregnancy, accompanied by a combination of abdominal pain, signs of hypovolemia, a decrease in haemoglobin levels, and foetal distress. This condition carries a significant risk of maternal and perinatal mortality and morbidity [5]. Due to the absence of comprehensive global surveillance, estimating the exact incidence of SHiP remains challenging. However, a study conducted by the Italian Obstetric Surveillance System (ItOSS) between 2013 and 2017 documented seven maternal deaths attributed to SHiP and calculated a specific maternal mortality rate (MMR) of 0.2 cases per 100,000 live births [6]. While the precise aetiology of SHiP remains unclear, advanced maternal age, endometriosis [7], multiple pregnancies and assisted reproductive technologies (ART) have been suggested as potential risk factors [8, 9].

The case we are about to present is unique in its kind, as it is a case of spontaneous hemoperitoneum in pregnancy in a patient with previous caesarean section. This case underlines the importance of making a correct differential diagnosis, because in a pregnant patient with previous uterine surgery, the onset of abdominal pain and the presence of intra-abdominal fluid do not necessarily mean uterine rupture.

CASE PRESENTATION

We present the case of a 38-year-old woman, G3P1, with a previous obstetric history of a full-term caesarean section for arrested labour, pregnant at 29 weeks of gestation with a sudden onset of acute abdominal pain.

Ultrasound performed upon admission showed a moderate amount of free fluid collection in both the hepatic and splenic recesses, extending into the pelvis. At the level of the lower uterine segment, in the site of a previous caesarean section scar, there are no apparent signs of dehiscence or rupture of the uterine wall. Blood tests revealed a haemoglobin level of 9.2 g/dL.

Twelve hours after the previous tests, a blood sample was repeated and attested a sudden drop of the haemoglobin level at 8.3 g/dL. A repeated ultrasound revealed diffuse abdominal free fluid collection, increased in volume compared to the previous imaging documentation, with echogenicity compatible with a haemorrhagic type of fluid collection.

It was decided to administer antenatal corticosteroid prophylaxis and foetal neuroprotection with magnesium sulphate. Due to persistent acute abdominal pain, anaemia with signs of initial hemodynamic instability in a suspected case of hemoperitoneum, an urgent surgical intervention was decided. A diagnostic laparoscopy was performed to investigate intra-abdominal bleeding. Blood clots were found in the parietocolic gutters, hepatic lodge, and splenic lodge and about 1,300 mL of blood in the abdominal cavity was evacuated. No active bleeding from the upper abdomen seemed to be present. However, the gravid uterus impeded adequate visualization of the lower abdomen, preventing identification of the bleeding source.

Therefore, a decision was made to convert to laparotomy based on the haemodynamic instability of the patient and the confirmed significant hemoperitoneum (> 1,000 cc) found on laparoscopic approach. Upon open exploration, no active upper abdominal bleeding was confirmed. While the pelvic cavity was thoroughly examined, no signs of uterine rupture or bleeding from previous scar sites were detected. The laparotomic surgical approach allowed for a complete visualization of the pelvis, leading to the identification of the active bleeding focus: a 2-cm firm clot with surrounding inflammation and active bleeding in the middle third of the left fallopian tube. Additionally, a secondary bleeding site was found on the omentum. Due to the patient's deteriorating hemodynamic status, an emergency caesarean section was decided.

A transplacental transverse corporal incision was performed, and a female newborn was extracted. The neonate weighed 1,237 grams and had an APGAR score of 1/4/7, umbilical cord pH was 7.30 with a BE of -2.3 mMol/L. The newborn required intubation and ventilation, and surfactant was administered. The newborn was subsequently extubated at 6 hours of life with a good neonatal outcome. After the caesarean section, a salpingectomy and a partial omentectomy were therefore performed. No other sources of bleeding were evident. The histological results were unexpected, demonstrating foci of endometriosis in the fallopian tube, even though there was no previous medical history suggestive of endometriosis.

DISCUSSION

Hormonal changes during pregnancy can exert a substantial impact on the endometriotic tissue, in a process termed "decidualization" of ectopic endometrium, ultimately increasing the likelihood of bleeding from endometriotic lesions [10].

This phenomenon of decidualization explains why endometriosis is a major risk factor for SHiP. Unfortunately, endometriosis is still an underdiagnosed and underestimated condition, as evidenced by the case presented of an incidental diagnosis of endometriosis following a case of spontaneous hemoperitoneum of pregnancy.

In our case, the most obvious differential diagnosis was the previous caesarean section scar rupture but that was ruled out by a sonographic evaluation of the lower uterine segment. In our Clinic we routinely perform an ultrasound evaluation of the lower uterine segment (LUS) in pregnant women with a history of caesarean delivery. We use state-of-the-art ultrasound equipment and highly trained sonographers to perform LUS evaluations. Acquiring a high level of expertise in ultrasound evaluation of the lower uterine segment is essential for optimal maternal and foetal care in pregnant women with a previous caesarean section. Our team has extensive experience in interpreting ultrasound images and can identify even subtle abnormalities [11]. The expertise in sonographic LUS evaluation allows to accurately assess the thickness and integrity of the LUS and identify or rule out potential complications such as uterine rupture and dehiscence of a previous caesarean scar.

CONCLUSIONS

Spontaneous hemoperitoneum of pregnancy (SHiP) is a critical differential diagnosis in cases of acute abdominal pain during pregnancy. It should be considered especially in women with risk factors like endometriosis or those who have undergone assisted reproductive technologies (ART). However, as this case demonstrates, SHiP can occur even in the absence of known risk factors, and therefore, it should always be considered as a possible cause of acute abdominal pain in pregnant women, regardless of their medical history, and even if other conditions, like a ruptured caesarean scar, seemed more likely.

Early and accurate differential diagnosis is essential, as the therapeutic approach differs significantly from SHiP to uterine rupture. While with uterine rupture always necessitates a caesarean section, in cases of SHiP, in the presence of maternal hemodynamic stability and after identifying and controlling the bleeding site, it is possible, in some cases, to continue the pregnancy [12].

COMPLIANCE WITH ETHICAL STANDARDS

Authors' contribution

S.R.: Conceptualization. S.O.: Data curation. L.D., G.V.: Supervision. G.B.: Writing – original draft. M.A.: Writing – review & editing.

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N/A.

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Vitamin D supplement in pregnancy - dose and recommendations: a systematic review

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ABSTRACT

Objective. This systematic review synthesises evidence from randomised controlled trials (RCTs) on the safety, efficacy, and optimal dosing of vitamin D supplementation during pregnancy.

Materials and Methods. The present study is an integrative review of literature based on clinical trials published in journals indexed in the PubMed databases and the Cochrane Library. A comprehensive search was conducted in PubMed and the Cochrane Library until May 2024. We included RCTs evaluating vitamin D supplementation in pregnant women, focusing on maternal and neonatal outcomes and dosing strategies. A total of 64 RCTs were thematically synthesised using PRISMA guidelines.

Results. Evidence from 64 RCTs indicates that vitamin D supplementation is associated with improved maternal 25(OH) D levels, especially when administered at daily doses of ≥ 2,000 IU. Supplementation of 4,000 IU/day was found to be safe and most effective in achieving maternal and neonatal sufficiency. Higher intermittent doses (*e.g.*, 50,000 IU every two weeks) also showed safety and improved metabolic profiles in some populations. While supplementation did not consistently reduce preeclampsia or gestational diabetes mellitus, it was associated with a lower risk of atopic eczema in offspring during the first year of life and improved bone mineralisation in children up to six years of age.

Conclusions. Vitamin D supplementation during pregnancy is safe and effective in correcting deficiency, particularly at doses ≥ 2,000 IU/day. Although clinical benefits on major obstetric outcomes remain inconsistent, supplementation may offer advantages in select populations. Defining baseline vitamin D status, ethnicity, and timing of supplementation remains crucial when determining optimal dosing strategies.

INTRODUCTION

Vitamin D: physiology and source

Vitamin D is a fat-soluble, lipophilic prohormone proven to have many metabolic and biological functions. This vitamin comes primarily from exposure to sunlight and is found naturally only in a few foods, such as fish-liver oils, fatty fish, mushrooms, egg yolks, butter, and liver. There are two physiologically active forms of vitamin D collectively called calcife-

rol: D2 (ergocalciferol) and D3 (cholecalciferol). Plants synthesise vitamin D2 while vitamin D3 is produced in humans upon skin exposure to ultraviolet light B (UVB) radiation [1, 2]. The foetus is entirely dependent on maternal sources of vitamin D [2, 3].

Importance of vitamin D

The classical role of vitamin D is in calcium and phosphate homeostasis, which is required for bone mineralisation [4, 5]. However, vitamin D is a crucial modulator of many essential biological effects. The actions of vitamin D metabolites are mediated by the vitamin D receptor (VDR), which is expressed in most tissues. The VDR has been shown to regulate cellular differentiation and target gene expression in many cell types, including the immune system [5, 6].

Vitamin D in pregnancy: effect on pregnancy outcome and recommendations for its use

In pregnancy, maternal vitamin D status has been linked to many health outcomes in the mother and offspring. During pregnancy, there are significant alterations in phosphate and calcium metabolism owing to calcium accumulating in the foetal skeleton, and as the foetus relies exclusively on the maternal supply of vitamin D, which it receives across the placenta, a low level of vitamin D during the pregnancy and especially during the early stage of pregnancy produce less bone mineral content in the foetal skeleton [2]. A wealth of studies has reported on other obstetric outcomes and complications, including preeclampsia, gestational diabetes, and mode and timing of delivery. Also, many foetal and childhood outcomes are linked to maternal vitamin D status, including measures of foetal size, and later childhood outcomes, such as asthma [4]. As a result, several national and international guidelines recommend vitamin D supplementation during pregnancy. Most recommend 10-15 micrograms (400-600 IU) of vitamin D daily throughout pregnancy, although this is not currently supported by the World Health Organisation (WHO) [1]. The Food and Nutrition Board at the Institute of Medicine of the National Academies suggests that the recommended dietary allowance (RDA) of vitamin D in pregnancy and lactation is 15 micrograms (600 IU), with an estimated average requirement (EAR) of 10 micrograms (400 IU) and tolerable upper intake level of 100 micrograms (4,000 IU). The Endocrine Society recommends empiric vitamin D supplementation in dosages ranging from 15-125 micrograms (600-5,000 IU) daily for a healthy individual during pregnancy [5, 6].

Vitamin D deficiency: impact of the problem

Vitamin D deficiency is a major public health concern, is widespread among the general population, and pregnant women are a special important group. During pregnancy, the mother's nutrition intake needs to meet both their own nutritional needs and the needs of their developing foetus that is why vitamin D deficiency is highly prevalent in pregnant women; it is found in 60-80% of pregnant women with the highest deficiencies seen in the Middle East [7-9]. Given the high rates of vitamin D deficiency among pregnant women and possible effects on obstetric and offspring outcomes, a review of articles on using vitamin D supplementation in pregnancy was conducted to help inform future practice guidelines. Even though previous systematic reviews have reported on the role of prenatal vitamin D in birth outcomes, the clear evidence for its recommendation and the dose required is poorly understood. The primary purpose of this review is to map the evidence on the effects of vitamin D supplementation on pregnancy outcomes and the preferred dosage used in pregnancy to provide us with a comprehensive understanding of this issue.

MATERIALS AND METHODS

Literature search

The present study is a systematic review based on clinical trials published in journals indexed in the PubMed database and Cochrane Library. The goal strategy of the search was to identify studies that reported the associations between vitamin D supplementation in pregnancy and different aspects of pregnancy outcomes. The basic research strategy was developed for PubMed and modified as required for the Cochrane Library. A literature search was conducted using the following keywords: (((vitamin D) AND (supplement) AND (pregnancy)) AND (outcome). Filters were applied to free full-text and clinical trials.

We searched these databases for eligible articles for inclusion from inception until May 2024; abstracts and unpublished studies were not included. We included randomised controlled trials (RCTs), which adopted the Patient Intervention Comparison Outcome structure. The articles were selected from the search in the databases, from the reading

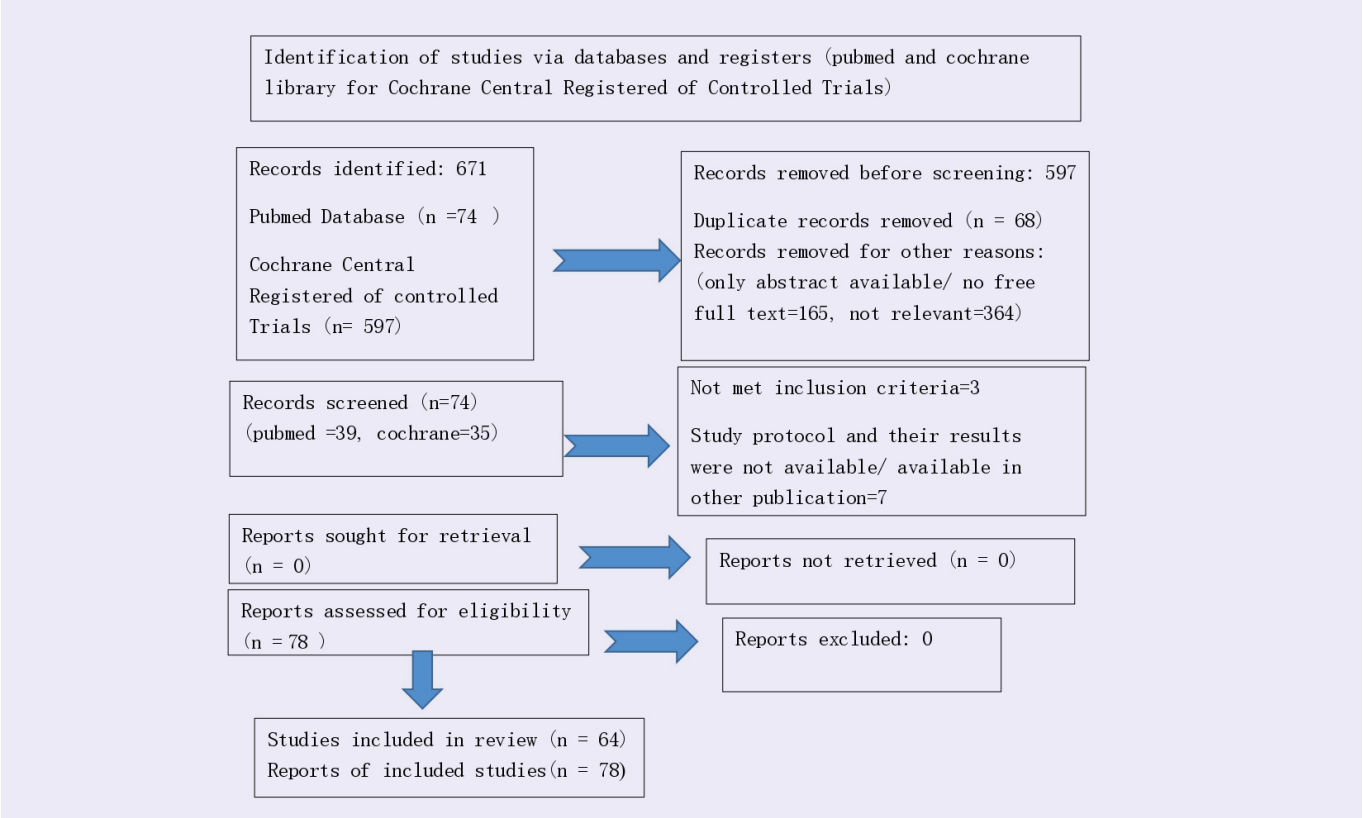

Figure 1. *PRISMA flow diagram.*

and selection of titles and abstracts, to the full selection of articles. The search was limited to human subjects and English language articles. The preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines were followed.

Inclusion and exclusion criteria

Studies were selected only if they satisfied the following criteria:

1. Original research article
2. Available in English.
3. Study carried out on humans (pregnant women of any gestational age)
4. The exposure of interest is vitamin D supplementation during pregnancy, irrespective of dose, duration, or time of commencement. Eligible intervention groups included daily or single-intermitted vitamin D supplementation (vitamin D2 or D3), alone or in combination with calcium and/or other micronutrients. Control groups included nutrients, no treatment, or a placebo.
5. The main outcomes of interest are the incidence of maternal morbidity and pregnancy outcome, as well as the safety of the dose used.

The exclusion criteria were as follows:

1. Research that did not supplement pregnant women.
2. Observational designs.
3. Review articles, letters to the editor, editorial materials, meeting abstracts, comments, case reports, and editorial notes.
4. Research in non-humans.
5. Studies that could not isolate the effects of vitamin D supplementation or intake.
6. Topics unrelated to the review.

Study selection and data extraction

The included studies were reviewed. One reviewer initially reviewed titles and abstracts. In the first phase, duplicates, trials available as abstract only, and not relevant topics were excluded. An in-depth review of full papers follows this. Then two review authors independently 1) assessed the eligibility of trials against the inclusion criteria, 2(extracted data from included trials, and 3) assessed the risk of bias in the included trials. The detailed steps of the study selection are given as a PRISMA flow diagram in **Figure 1**.

From all the eligible studies, the authors extracted the following information in a standard format: the first author's last name, year of publication, country where the study was performed, descriptions of the study design, number of participants, intervention details, primary outcome of the study, and its report. Pertinent information is summarised in **Table 1**.

Table 1. *Study characteristics.*

Study no.	Author and reference no.	Year of publication.	Country	Type of study	No. of participant.	Information about vitamin D intervention	Aim/Primary outcome	Report
1	Roth (10)	2018	Canada	RCT	1,300	Vitamin D3: (A) 0 IU/week, (B) 4,200 IU/week, (C) 16,800 IU/week, or (D) 28,000 IU/week in pregnancy, all with 0 IU/week postpartum; or (E) 28,000 IU/week in prenatal and postpartum periods	The primary outcome was length-for-age z-score at one year of age according to World Health Organization child growth standards.	*In a population with widespread prenatal vitamin D deficiency and fetal/infant growth restriction, maternal vitamin D supplementation from mid-pregnancy until birth or 6 months postpartum does not influence fetal or infant growth and has no beneficial or harmful effects on numerous other birth and infant outcomes.
2	Bhowmik (11)	2021	Bangladesh	RCT	748	*Oral dose of 200,000 IU of vitamin D every 60- 90 days. *Oral dose of 200 mcg of B12 every 14 days, equivalent to 14 mcg daily.	Evaluation of maternal and delivery outcomes, including adverse outcomes.	*Oral supplementation of high dose intermittent vitamin D or low dose vitamin B12 regime failed to correct the relevant nutritional deficiencies in Bangladeshi pregnant women as per protocol. Both dietary supplementation and high dose vitamin D corrected severe vitamin deficiency. *No significant difference in any of the pregnancy or birth outcomes was observed between three groups.
3	El-Heis (12)	2022	UK	RCT (2°ry analysis)	703	1000 IU vitamin D3/day.	To examine the influence of maternal cholecalciferol supplementation during pregnancy on the risk of atopic eczema in the offspring at ages 12, 24 and 48 months.	Maternal supplementation with cholecalciferol 1,000 IU daily from 14 weeks' gestation to delivery led to a reduced incidence of atopic eczema in the first year of life.
4	Jamilian (13)	2019	Iran	RCT	60	200 IU vitamin D (with other micro-nutrients) twice a day.	To determine the effects of magnesium-zinc-calcium-vitamin D co-supplementation on parameters of inflammation and oxidative stress, and pregnancy outcomes among women with gestational diabetes mellitus (GDM).	*Magnesium-zinc-calcium-vitamin D co-supplementation for 6 weeks to women with GDM may reduce biomarkers of inflammation and oxidative stress.
5	Rodgers (14)	2023	South Carolina	RCT (post hoc analysis)	156	400, 2,000, or 4000 IU vitamin D3/day	To determine whether there is a relationship between 25(OH)D concentration and child neuro development.	*Higher 25(OH)D concentrations early in life and higher doses of maternal vitamin D supplementation during pregnancy may have a positive association with neurodevelopment-al outcomes. *The vitamin D binding protein (VDBP) genotype is associated with neurodevelopment and differentially affects various fields of neurodevelopment.
6	Han (15)	2023	UK, Singapore, and New Zealand	RCT (2°ry analysis)	338	Daily dose of vitamin D3 400 IU (10 mcg) in addition to vitamins B2, B6, B12, as well as zinc, myo-inositol, and probiotics.	To investigate the effects of maternal supplementation from preconception throughout pregnancy until birth on human milk (HM) concentrations of vitamin D3 and B-vitamins.	Maternal supplementation during preconception and pregnancy increased HM vitamin D, but not B-vitamin concentrations in lactation.
7	Mirzakhai (16)	2016	Canada	RCT	876	4,400 vs. 400 IU vitamin D3/day	To assess the effect of vitamin D supplementation (4,400 vs. 400 IU/day), initiated early in pregnancy (10–18 weeks), on the development of preeclampsia.	*Vitamin D supplementation initiated in weeks 10–18 of pregnancy did not reduce preeclampsia incidence in the intention-to-treat paradigm. However, vitamin D levels of 30 ng/ml or higher at trial entry and in late pregnancy were associated with a lower risk of preeclampsia. *Differentially expressed vitamin D–associated transcriptomes implicated the emergence of an early pregnancy, distinctive immune response in women who went on to develop preeclampsia.

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8	Godfrey (17)	2023	UK, Singapore, and New Zealand	RCT (pre-pecified 2°ry analysis)	512	Daily dose of vitamin D3 400 IU (10 µg) in addition to vitamins B2, B6, B12, as well as zinc, myo-inositol, and probiotics.	To determine the influence of vitamin supplementation on longitudinal patterns of maternal vitamin status from preconception, through early and late pregnancy, to 6 months postdelivery.	Preconception and pregnancy supplementation in amounts available in over-the-counter supplements substantially reduced the prevalence of deficiency/ depletion markers before and during pregnancy, and a higher maternal plasma vitamin B12 was maintained during the recommended lactational period.
9	Ku (18)	2023	Singapore	RCT	227	400 IU/ 800 IU vitamin D3 daily.	To investigate whether intervention with an 800 IU oral vitamin D3 supplement compared to a 400 IU of vitamin D3 standard prenatal multivitamin, taken from early pregnancy until delivery, would lead to improved maternal serum 25OHD levels, lipid profiles, and pregnancy outcomes in women with overweight/ obesity (OW/OB) during pregnancy.	800 IU/day of vitamin D3 supplementation effectively increased the 25OHD levels and improved the vitamin D sufficiency status in pregnant women with OW/OB in pregnancy. However, no effect on lipid profiles or pregnancy outcomes were found.
10	Barker (19)	2017	UK	RCT (2°ry analysis)	203	1000 IU vitamin D3/day.	To determine the relationship between self-efficacy, compliance with study protocol and outcome.	*The differences in self-efficacy influence trial outcomes. Such information may help clinicians anticipate responses to routine vitamin D supplementation in pregnancy and identify those who may need more support to comply.
11	Harreiter (20)	2022	UK, Ireland, Austria, Poland, Italy, Spain and Belgium.	RCT (2°ry analysis)	154	1600 IU vitamin D3/day.	To assess the effects of a vitamin D supplementation with 1600 IU Vitamin D3/ day starting in early pregnancy versus placebo on maternal and fetal lipid parameters, body fat distribution as well as pregnancy outcomes.	*Vitamin D supplementation with 1600 IU/day vitamin D3 in overweight/obese pregnant women led to a safe and significant increase of vitamin D levels in pregnancy and high prevalence of vitamin D sufficiency without safety issues. *No significant differences between the treatment arms in maternal lipid parameters nor in fetal lipid parameters or birth outcomes were found.
12	Sudfeld (21)	2022	Tanzania	RCT	2300	3000 IU vitamin D3/ day	The primary outcomes were (i) maternal HIV progression or death, (ii) small for-gestational-age (SGA) live births (<10th percentile), and (iii) infant stunting at 1 year of age (length-for-age z-score <−2).	*Maternal vitamin D3 supplementation during pregnancy and lactation did not significantly affect the risk of maternal HIV disease progression or death, SGA live births, or infant stunting at 1 year of age.
13	Morris (22)	2021	Bangladesh	RCT	1174	4,200, 16,800, and 28 000 IU vitamin D per week.	To examine the effect of maternal vitamin D supplementation during pregnancy and lactation on risk of acute respiratory infection (ARI) in infants up to 6 months of age in Bangladesh.	*Despite a high prevalence of maternal baseline vitamin D deficiency and significant effects of maternal vitamin D supplementation on infant vitamin D status, the intervention did not reduce the risk of microbiologically confirmed ARI in infants up to 6 months of age.
14	Alhomaid (23)	2021	Northen Ireland	RCT	240	10 or 20 µg vitamin D3/ day.	To examine the effects of maternal supplementation of 10 µg compared with 20 µg vitamin D3/ day on maternal and umbilical cord 25(OH) D level.	*Supplementation of 20 µg vitamin D3/d is needed to attain maternal and umbilical cord 25(OH)D concentrations ≥50 nmol/L.
15	Callaghan (24)	2022	Bangladesh	RCT (2°ry analysis)	642	4,200/0, 16,800/0, 28,000/0, or 28,000/28,000 IU vitamin D3/week	To examine hypothesized effects of improvements in early-life vitamin D status on childhood musculoskeletal health.	*Maternal prenatal, with or without postpartum, vitamin D supplementation does not improve child bone mineral content (BMC), areal bone mineral density (aBMD), or grip strength at 4 y of age.

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16	Hornsby (25)	2017	UK	RCT	51	400/ 4,400 IU/day vitamin D3	To investigate the effect on neonatal immunity of maternal supplementation with 4,400 IU/day vitamin D3 during the second and third trimesters of pregnancy.	*Vitamin D supplementation during pregnancy modifies the immune system of the neonate. This modified immune system might be better equipped to protect the host against pathogenic infections.
17	Hollis (26)	2011	South Carolina	RCT	350	400, 2000 or 4000 IU vitamin D3/ day.	Maternal/ neonatal circulating 25(OH)D level at delivery.	*Vitamin D supplementation of 4000 IU/day for pregnant women is safe and most effective in achieving sufficiency in all women and their neonates regardless of race, whereas the current estimated average requirement is comparatively ineffective at achieving adequate circulating 25(OH)D concentrations, especially in African Americans.
18	Goldring (27)	2013	UK	RCT	180	800 IU ergocalciferol daily until delivery or single oral bolus of 200,000 IU cholecalciferol	To examine the effect of prenatal vitamin D on childhood wheezing.	*Prenatal vitamin D supplementation in late pregnancy that had a modest effect on cord blood vitamin D level, was not associated with decreased wheezing in offspring at age three years.
19	Curtis (28)	2021	UK	RCT (2°ry analysis)	372	1000 IU vitamin D3/ day.	The influence of vitamin D supplementation in pregnancy on maternal postnatal bone indices.	*Maternal urinary C-terminal telopeptide of type I collagen (CTX), a bone resorption marker, rises through pregnancy, although to a lesser degree with gestational cholecalciferol supplementation, and is inversely associated with maternal bone mass postpartum.
20	Schulz (29)	2017	South Carolina	RCT	43	400 IU/ 4,400 IU of vitamin D3/ day	To investigate the role of maternal 25(OH)D, the nutritional indicator of vitamin D status, in relation to placental maintenance and, specifically, expression of placental gene targets related to angiogenesis and vitamin D metabolism.	*A significant association between maternal vitamin D status and the expression of Soluble FMS-like tyrosine kinase 1 (sFlt-1) and vascular endothelial growth factor (VEGF) at the mRNA level were reported. Achieving maternal circulation of 25(OH)D 100 nmoles/L suggests the impact of maternal vitamin D3 supplementation on gene transcription in the placenta, thereby potentially decreasing antiangiogenic factors that may contribute to vascular pregnancy complications.
21	Karamali (30)	2016	Iran.	RCT	60	1,000 mg Ca/day and two pearls containing 1,250 µg (50 000 IU) of vitamin D3 during the intervention (one at study baseline and another at day 21 of the intervention)	To assess the effects of Ca + vitamin D supplementation on pregnancy outcomes in women with gestational diabetes mellitus (GDM).	*Ca + vitamin D supplementation for 6 weeks among pregnant women with GDM led to decreased caesarean section rate and maternal hospitalization, and decreased macrosomia, hyperbilirubinaemia and hospitalization in newborns.
22	Rodrigues (31)	2021	Brazil	RCT (feasibility trial)	69	(1) fortified sachet (vitamin D3 (500 IU) and calcium) and powdered milk plus periodontal therapy during pregnancy (early PT); (2) placebo sachet and powdered milk plus early PT; (3) fortified sachet and powdered milk plus late PT (after delivery); (4) placebo sachet and powdered milk plus late PT.	To explore the effect of a non-pharmaceutical multi-component intervention on periodontal health and metabolic and inflammatory profiles among pregnant women with periodontitis receiving prenatal care in a Brazilian public health centre.	*The mean BOP (% sites with bleeding on probing) was significantly reduced in the early PT groups, while BOP worsened in the late PT groups. No significant effect of fortification on BOP was observed. Changes in glucose levels and variation on birthweight did not differ among groups
23	Cooper (32)	2016	UK	RCT (2°ry analysis)	737	1,000 IU vitamin D3/ day	Body bone mineral content (BMC) of the neonate.	*Supplementation of mothers with 1,000 IU/day cholecalciferol during pregnancy did not lead to increased offspring whole body BMC compared with placebo.

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24	Braithwaite (33)	2019	UK	RCT (2°ry analysis)	195	Vitamin D3 1000 IU/ day from ~14 weeks gestation.	To identify whether antenatal vitamin D3 supplementation affects iron status (via hepcidin suppression) and/or inflammation.	*Vitamin D3 supplementation had no effect on hepcidin, ferritin, or inflammatory status suggesting no adjunctive value of vitamin D3 in reducing rates of antenatal iron deficiency.
25	Moon (34)	2016	UK	RCT	829	1,000 IU vitamin D3/ day.	To assess which maternal and environmental characteristics were associated with better 25(OH)D level following vitamin D supplementation.	*Women who gain more weight during pregnancy, have lower 25(OH)D in early pregnancy, or deliver in winter tend to achieve a lower 25(OH)D in late pregnancy when supplemented with 1,000-IU/day cholecalciferol than do women with the converse attributes.
26	Motamed (35)	2019	Iran	RCT	84	1000 / 2000 IU vitamin D/day.	To evaluate the efficacy of two doses of vitamin D3 supplementation during pregnancy on maternal and cord blood vitamin D status, inflammatory biomarkers, and maternal and neonatal outcomes.	*Supplementation with 2,000 IU/day vitamin D3 instead of 1000 IU/day from the first trimester of pregnancy could be more effective in improving vitamin D status of the pregnant women and decreasing pro-inflammatory cytokines including TNF-α in mother and IL-6 in cord blood. *Also supplementation with 2000 IU/day vitamin D3 helps improve neonatal outcomes including the birth sizes (weight, length, and head circumference) compared with 1000 IU/day group.
27	Kabuyanga (36)	2024	Democratic Republic of Congo	RCT	1,159	A single monthly dose of vitamin D3 (60,000 IU) orally for 6 months.	To evaluate the effect of cholecalciferol supplementation on the incidence of preeclampsia in primigravid women and its related maternal and foetal outcomes.	*A single monthly dose (60,000 IU) of vitamin D supplementation, started in early pregnancy, significantly reduced the incidence of preeclampsia and its maternal and foetal complications.
28	Litonjua (37)	2020	USA	RCT (2°ry analysis) VDAART	806	4400 IU of vitamin D3 per day	To determine whether children born to mothers who had received 4400 IU of vitamin D3/ day during pregnancy (vitamin D group) would have a lower incidence of asthma and recurrent wheeze at the age of 6 years.	*Vitamin D supplementation during the prenatal period alone did not influence the 6-year incidence of asthma and recurrent wheeze among children who were at risk for asthma.
29	Enkhmaa (38)	2018	Mongolia	RCT	344	600, 2,000, or 4,000 IU vitamin D3	To test the impact of different concentrations of vitamin D supplementation on 25(OH)D concentrations of pregnant women in Mongolia.	*Daily supplementation of 4000 IU during pregnancy is safe and achieved higher maternal and neonatal 25(OH)D concentrations than 2000 IU. Daily 600 IU supplements are insufficient to prevent vitamin D deficiency in Mongolia.
30	Vaziri (39)	2016	Iran	RCT	136	2,000 IU vitamin D3/ day.	To evaluate the effect of vitamin D3 supplementation on perinatal depression scores.	*Consuming 2,000 IU vitamin D3 daily during late pregnancy was effective in decreasing perinatal depression levels.
31	Moon (40)	2017	UK	RCT	682	1,000 IU/ day vitamin D3 from 14 weeks of gestation until delivery.	To determine whether single-nucleotide polymorphisms (SNPs) in DHCR7, CYP2R1, CYP24A1, and GC genes are associated with the response to gestational cholecalciferol supplementation.	*Common genetic variation is associated with baseline 25(OH)D in pregnancy and the response to antenatal supplementation with 1,000 IU/day cholecalciferol, but with differing SNPs appearing to be important before and after supplementation. (Our findings suggest that analysis of SNPs may have an important role in identifying high-risk categories of individuals who are likely to require higher doses of vitamin D to achieve repletion).
32	Wagner (41)	2013	South Carolina	RCT	161	400, 2,000, or 4000 IU vitamin D3/ day	To determine whether 4,000 IU vitamin D3/ day (vs. 2,000 IU/day) during pregnancy is safe and improves maternal/neonatal 25(OH)D in a dose-dependent manner.	*Maternal supplementation with 2,000 and 4,000 IU vitamin D/day during pregnancy improved maternal/neonatal vitamin D status. *Evidence of risk reduction in infection, preterm labor and preterm birth was suggestive, requiring additional studies powered for these endpoints.

Study no.	Author and reference no.	Year of publication.	Country	Type of study	No. of participant.	Information about vitamin D intervention	Aim/Primary outcome	Report
33	Litonjua (42)	2016	USA	RCT	806	daily 4,000 IU vitamin D plus a prenatal vitamin containing 400 IU vitamin D/a placebo plus a prenatal vitamin containing 400 IU vitamin D.	To determine whether prenatal vitamin D supplementation can prevent asthma or recurrent wheeze in early childhood.	*In pregnant women at risk of having a child with asthma, supplementation with 4,400 IU/day of vitamin D compared with 400 IU/day significantly increased vitamin D levels in the women. *The incidence of asthma and recurrent wheezing in their children at age 3 years was lower by 6.1%, but this did not meet statistical significance; however, the study may have been underpowered.
34	Zerofsky 43))	2016	California	RCT	51	400/ 2000 IU vitamin D3/ day from < 20 week to delivery	To assess the effects of vitamin D supplementation during pregnancy on vitamin D status and markers of immune function associated with adverse pregnancy outcomes.	*Supplementation with 2000 IU/day is more effective at increasing vitamin D status in pregnant women than 400 IU/day and is associated with increased regulatory T cell immunity that may prevent adverse outcomes caused by excess inflammation.
35	Roth (44)	2012	Bangladesh	RCT	61	A single oral vitamin D3 dose (70,000 IU)	To assess the change in serum 25-hydroxyvitamin D concentration over time, estimated using model-independent pharmacokinetic parameters.	*The response to a single 70,000 IU dose of vitamin D3 was similar in pregnant and non-pregnant women in Dhaka and consistent with previous studies in non-pregnant adults. These preliminary data support the further investigation of antenatal vitamin D3 regimens involving doses of ≤70,000 IU in regions where maternal-infant vitamin D deficiency is common.
36	Roth (45)	2013	Bangladesh	RCT	160	35,000 IU/week of vitamin D3	To evaluate the effect of high-dose prenatal 3rd trimester vitamin D3 supplementation on maternal and neonatal (cord blood) serum (25(OH)D) concentration (primary biochemical efficacy outcome) and maternal serum calcium concentration (primary safety measure).	*Antenatal 3rd-trimester vitamin D3 supplementation (35,000 IU/week) significantly raised maternal and cord serum 25(OH)D concentrations above 50 nmol/L in almost all participants without inducing hypercalcemia or other observed safety concerns. *Doses up to 35,000 IU/week may be cautiously used in further research aimed at establishing the clinical effects and safety of vitamin D3 supplementation in pregnancy.
37	Roth (46)	2013	Bangladesh	RCT	28	70,000 IU once + 35,000 IU/week vitamin D3 or 14,000 IU/week vitamin D3	To assess the biochemical dose-response and tolerability of high-dose prenatal vitamin D3 supplementation.	*A regimen of an initial dose of 70,000 IU and 35,000 IU/week vitamin D3 in the third trimester of pregnancy was non-hypercalcemic and attained [25(OH)D] ≥ 80 nmol/L in virtually all mothers and newborns.
38	Newton (47)	2022	South Carolina.	RCT	74	400 IU/ 6400 IU vitamin D3/day	To determine the effects of maternal vitamin D sufficiency on infant plasma concentrations of (25(OH)D) and 11 cytokines.	*Maintenance of maternal vitamin D sufficiency during pregnancy likely continues to affect the health of breastfed infants.
39	Han (48)	2023	Southampton-on (UK), Singapore, and Auckland (New Zealand).	RCT	338	Daily dose of vitamin D3 400 IU (10 µg) in addition to vitamins B2, B6, B12, as well as zinc, myo-inositol, and probiotics.	To investigate the effects of maternal supplementation from preconception throughout pregnancy until birth on human milk (HM) concentrations of vitamin D3 and B-vitamins and to characterise longitudinal changes in milk concentrations of these vitamins.	*Maternal supplementation during preconception and pregnancy increased HM vitamin D, but not B-vitamin concentrations in lactation.
40	Wagner (49)	2013	South Carolina	RCT	504	400, 2000, or 4000 IU vitamin D3/day / 2000 or 4000 IU vitamin D3/day.	To assess the safety and health effects of vitamin D supplementation during pregnancy.	*Supplementation with 4,000 IU/day was associated with lower risk of hypovitaminosis D than Control and 2,000 IU groups. *While not statistically significant, there was a trend toward lower rates of comorbidity of pregnancy as supplementation dose increased

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41	Dawodu (50)	2013	UAE	RCT	162	400, 2000, and 4000 IU/day vitamin D3	To determine effectiveness and safety of prenatal 2000 IU and 4000 IU/day compared with 400 IU/day vitamin D3 supplementation in population in which vitamin D deficiency is endemic.	*Vitamin D supplementation of 2000 and 4000 IU/day appeared safe in pregnancy, and 4000 IU/day was most effective in optimizing serum 25(OH)D concentrations in mothers and their infants.
42	Harrington (51)	2014	Bangladesh	RCT	130	35,000 IU/week vitamin D3 from 26 to 29 wk of gestation.	To assess the effect of high-dose antenatal vitamin D supplementation on fetal and neonatal calcium concentrations.	*High-dose antenatal third-trimester vitamin D supplementation attenuated the early postnatal calcium nadir, without increasing the risk of postnatal hypercalcemia.
43	Hossain (52)	2014	Pakistan	RCT	193	4,000 IU vitamin D3 daily, started at 20 weeks and continued till delivery.	To determine whether vitamin D supplementation during pregnancy affects obstetric and neonatal outcomes.	*Maternal vitamin D supplementation improved maternal and neonatal vitamin D status. *The obstetric outcomes were comparable between the two groups. *One- and 5-minute Apgar scores were significantly higher in the intervention group Neonatal anthropometric parameters were comparable between the two groups.
44	Karamali (53)	2015	Iran	RCT	60	50 000 IU vitamin D3 every 2 weeks from 20 to 32 weeks of gestation.	To assess the beneficial effects of high-dose vitamin D supplementation on metabolic profiles and pregnancy outcomes among pregnant women at risk for pre-eclampsia.	*High-dose vitamin D administration among women at risk for pre-eclampsia had beneficial effects on insulin metabolism parameters, serum HDL-cholesterol, and plasma total antioxidant capacity concentrations, but did not affect fasting plasma glucose, other lipid profiles, inflammatory factors and other biomarkers of oxidative stress. *No significant effect of high-dose cholecalciferol supplementation on pregnancy outcome.
45	Asemi (54)	2015	Iran	RCT	45	50 000 IU vitamin D3 2 times during the study: at study baseline and day 21 of intervention.	To assess the effect of vitamin D supplementation on pregnancy outcomes of pregnant women with gestational diabetes mellitus (GDM) who were not on oral hypoglycemic agents.	*Vitamin D supplementation for 6 weeks among pregnant women with GDM resulted in decreased maternal polyhydramnios and infant hyperbilirubinemia compared with placebo.
46	Griffiths (55)	2015	UK	RCT	99	800 IU ergocalciferol daily until delivery, or a single oral bolus of 200,000 IU cholecalciferol	To examine the effect of prenatal Vitamin D on healthcare utilisation in the first three years of life.	* There is no evidence that prenatal vitamin D supplementation from 27 weeks gestation to delivery, at doses which failed to completely correct maternal vitamin D deficiency, influence overall healthcare utilisation in children in the first 3 year.
47	Singh (56)	2021	India	RCT	164	60,000 IU vitamin D3 oral tablet/ capsule weekly / 60,000 IU vitamin D3 injection intramuscular every fortnight for 8 weeks.	To study perinatal outcomes in vitamin D deficiency, and effect of oral and intramuscular vitamin D3 supplementation in antenatal women on pregnancy outcomes.	*There is a high prevalence of vitamin D deficiency in pregnant women in India. Supplementation of Vitamin D as a part of routine antenatal care needs to be established. *Both oral and intramuscular vitamin D are effective.
48	Stoutjesdijk (57)	2019	Netherlands	RCT	36	Increasing dose of daily vitamin D3 (10, 35, 60 and 85 µg) from 20 weeks gestation up to 4 weeks postpartum (PP).	To study the influence of daily 10–85 µg vitamin D supplements during pregnancy and lactation on maternal vitamin D status and mature milk antirachitic activity (ARA).	*Dosages of 35 µg vitamin D3/day or higher were needed to increase 25(OH)D to adequacy in >97·5% of participants at 36 weeks gestation, while >85 µg/d was needed to reach this target in >97·5% of participants at 4 weeks PP. *Milk ARA at 4 weeks PP increased in a dose-dependent manner.
49	Rostami (58)	2018	Iran	RCT	788	50,000 IU vitamin D3 oral weekly/ 300,000 IU vitamin D3 intramuscularly.	To determine the effectiveness of a prenatal screening program on optimizing [25(OH)D] levels and preventing pregnancy complications.	*A prenatal vitamin D screening and treatment program is an effective approach in detecting deficient women, improving 25(OH)D levels, and decreasing pregnancy adverse outcomes.

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50	Yap (59)	2015	Sydney	RCT	158	5,000 IU vitamin D3 daily/ 400 IU vitamin D3 daily	To investigate the effects of vitamin D supplementation on glucose metabolism during pregnancy.	*High dose vitamin D supplementation commencing at a mean of 14 weeks' gestation does not improve glucose levels in pregnancy. However, in women with baseline levels <32 ng/mL, 5,000 IU per day was well tolerated and highly effective at preventing neonatal vitamin D deficiency.
51	Mir (60)	2016	India	RCT	87	1,000 IU Vitamin D daily/ 30,000 IU Vitamin D monthly/2,000 IU Vitamin D daily/60,000 IU Vitamin D monthly.	To assess the efficacy and safety of various doses of vitamin D supplementation during pregnancy	Vitamin D supplementation with 2000 IU/day or 60,000 IU/month is very effective and safe in achieving Vitamin D sufficiency in pregnant women.
52	Wolsk (61)	2017	USA, Denmark	RCT		4,000 IU vitamin D3/day/2,400 IU vitamin D3/day.	To perform a combined analysis of two randomized controlled trials and investigate whether maternal (25(OH)D) level at trial entry modified the risk of asthma/recurrent wheeze from 0-3 years.	*The combined analysis shows that vitamin D supplementation during pregnancy results in a significant reduced risk of asthma/recurrent wheeze in the offspring, especially among women with 25(OH)D level 30 ng/ml at randomization, where the risk was almost halved.
53	Chawes (62)	2016	Denmark	RCT	581	Vitamin D3 (2,400 IU/day) from 24 weeks gestation to 1 week postpartum.	To determine whether supplementation of vitamin D3 during the third trimester of pregnancy reduces the risk of persistent wheeze in the offspring.	*The use of 2,800 IU/day of vitamin D3 during the third trimester of pregnancy compared with 400 IU/day did not result in a statistically significant reduced risk of persistent wheeze in the offspring through age 3 years. However, interpretation of the study is limited by a wide confidence interval (CI) that includes a clinically important protective effect.
54	Mirzaei-Azandaryanl (63)	2022	Iran	RCT	84	4,000 IU vitamin D3 daily.	To determine the effect of vitamin D supplementation on fasting blood glucose (FBG) levels, fasting blood insulin (FBI) levels and insulin resistance index (HOMA-IR) (primary outcomes) and symptoms of depression, musculoskeletal pain, frequency of gestational diabetes (GDM) and the frequency of abortion (secondary outcomes).	*Vitamin D could improve the musculoskeletal pain in pregnant women but couldn't decrease FBG, FBI, HOMA-IR, depression symptoms score, incidence of GDM and abortion.
55	Brustad (64)	2020	Denmark	RCT (2°ry analysis)	517	2800 IU/day (high-dose) vs 400 IU/d (standard-dose) from pregnancy week 24 until 1 week after birth.	To investigate the effect of a high dose vs standard dose of vitamin D supplementation in pregnancy on anthropometric and bone outcomes until age 6 years in the offspring.	*High-dose vitamin D supplementation in pregnancy improved offspring bone mineralization through age 6 years compared with the standard dose, suggesting an increased recommended gestational intake, which may influence peak bone mass, fracture risk, and risk of osteoporosis later in life. We found no supplementation effect on anthropometric outcomes.
56	Mojibian (65)	2015	Iran	RCT	470	400 IU vitamin D daily / 50,000 IU vitamin D every 2 weeks.	To assess the effects of 50,000 IU of vitamin D every two weeks supplementation on the incidence of gestational diabetes (GDM), gestational hypertension, preeclampsia and preterm labor, vitamin D status at term and neonatal outcomes contrasted with pregnant women that received 400 IU vitamin D daily.	*Consumption of 50,000 IU vitamin D every 2 weeks from 12 weeks of pregnancy until delivery significantly reduced the incidence of GDM.
57	Asemi (66)	2012	Iran	RCT	49	500 mg carbonate calcium plus 200 IU vitamin D3.	To determine the effects of consumption calcium-vitamin D supplements on metabolic profiles among Iranian pregnant women at risk for pre-eclampsia.	*Consumption of calcium-vitamin D supplements for 9 weeks during pregnancy among pregnant women at risk for pre-eclampsia resulted in decreased fasting plasma glucose (FPG) and serum triglycerides levels as compared to the placebo group, but could not affect serum total-, HDL-, LDL-cholesterol levels

Study no.	Author and reference no.	Year of publication.	Country	Type of study	No. of participant.	Information about vitamin D intervention	Aim/Primary outcome	Report
58	Sass (67)	2020	Denmark	RCT (2°ry analysis)	551	High-dose (i.e, 2800 IU/day) vs standard dose (i.e, 400 IU/day) vitamin D3 supplementation from pregnancy week 24 until 1 week after birth.	To determine whether high-dose vitamin D supplementation during pregnancy improves offspring neurodevelopment from birth to age 6 years.	*Maternal high dose vitamin D supplementation during the third trimester of pregnancy did not improve neurodevelopmental outcomes in the offspring during the first 6 years of life.
59	Motamed (68)	2020	Iran	RCT (2°ry analysis)	73	1,000 IU/2,000 IU/ day vitamin D.	To assess the efficacy of two dosages of vitamin D (1000 IU/day and 2000 IU/day) on certain metabolic parameters including glycaemic, lipidemic and parathyroid hormone as well as oxidative stress (OS) status.	*Supplementation with 2,000 IU/d vitamin D had no more beneficial effects on the studied biomarkers of glycemic, lipidemic and OS status of the maternal and cord blood than with 1,000 IU/day. Nevertheless, supplementation with 2,000 IU a day, compared with 1,000 IU/day, was more effective in improving vitamin D status and lowering the occurrence of suboptimal circulating calcidiol concentrations during pregnancy.
60	Nausheen (69)	2021	Pakistan	RCT	257	4,000 IU/day/ 2,000 IU/ day/ 400 IU/ day vitamin D3.	To evaluate the effect of different doses of vitamin D supplementation during pregnancy on biochemical markers (serum 25(OH)D, calcium, phosphorus and alkaline phosphatase) in women and neonates, and on pregnancy and birth outcomes (gestational diabetes, pre-eclampsia, low birth weight, preterm births and stillbirths).	*Vitamin D supplementation of 4,000 IU/day was more effective in reducing vitamin D deficiency among pregnant women and improving serum 25(OH)D levels in mothers and their neonates compared with 2,000 IU/day and 400 IU/day.
61	Nørrisgaard (70)	2019	Danemark	RCT (Post hoc analysis)	496	2,400 IU/day vitamin D3.	To assess the association of a high-dose vitamin D supplementation in pregnant women with enamel defects and caries in their offspring.	*High-dose vitamin D supplementation during pregnancy was associated with approximately 50% reduced odds of enamel defects in the offspring.
62	Valizadeh (71)	2016	Iran	RCT	84	200,000 IU vitamin D3 for each of the first two days, and then 50,000 IU per week thereafter, up to 700,000 IU in total.	To assess the impact of prenatal vitamin D supplementation on postpartum dysglycaemia in gestational diabetes mellitus (GDM) patients.	*Although the high vitamin D supplementation dose safely increases the serum 25OHD, in GDM cases, the higher dose does not affect the plasma glucose level or insulin resistance at short term follow-up after delivery.
63	Naghshineh (72)	2016	Iran	RCT	138	600 IU vitamin D daily at 16 week gestation until labor.	To investigate the association between vitamin D supplement and preeclampsia in pregnant women.	* Vitamin D supplementation during the third trimester of pregnancy; reduce the risk of preeclampsia however, this was not statistically significant.
64	Etemadifar (73)	2015	Iran	RCT	15	50,000 IU/week vitamin D3.	To assess the safety and efficacy of high-dose oral vitamin D3 supplementation during pregnancy in women with multiple sclerosis (MS) in Isfahan, Iran.	*Adding high dose vitamin D3 supplementation during pregnancy to routine care of women with MS had significant effect on the serum 25(OH)D levels, expanded disability status scale (EDSS) and number of relapse events during pregnancy and within 6 months after delivery.

Table 2. *Summary of dose–response categories of the studies included.*

Outcome Domain	Dose Group	No. of RCTs	Effect Observed	Representative Studies
Maternal 25(OH)D levels post-supplementation	<1,000 IU/day	~6	Often insufficient to correct the deficiency	Alhomaid (2021), Ku (2023), Moon (2016), Stoutjesdijk (2019), Dawodu (2013), Nausheen (2021)
Maternal 25(OH)D levels post-supplementation	1,000–2,000 IU/day	~7	Moderate efficacy; improves status in non-deficient women	Motamed (2019), Enkhmaa (2018), Mir (2016), Godfrey (2023), Harreiter (2022), Vaziri (2016), Wagner (2013)
Maternal 25(OH)D levels post-supplementation	>2,000 IU/day	~9	Most effective in correcting deficiency; consistently raised maternal and neonatal levels	Roth (2018, 2013), Hollis (2011), Dawodu (2013), Nausheen (2021), Enkhmaa (2018), Wagner (2013), Mirzakhani (2016), Kabuyanga (2024),
Maternal 25(OH)D levels post-supplementation	Bolus doses (e.g., ≥50,000 IU every 2 weeks or monthly)	~5	Showed safety and improved vitamin D status, but requires more study	Mojibian (2015), Singh (2021), Rostami (2018), Naghshineh (2016), Yap (2015)

We also noted limitations and possible bias, such as small samples, suboptimal exposure, outcome measures, or other indications of poor study quality (for example, inadequate consideration of confounders).

RESULTS

Overview of included studies

A total of 64 randomised controlled trials (RCTs) were included in this systematic review. These studies were published between 2011 and 2024 and were conducted across diverse geographic regions, including North America, Europe, South Asia, the Middle East, and Sub-Saharan Africa. Intervention doses ranged from 400 IU/day to 50,000 IU every two weeks, with some studies using bolus dosing strategies. Outcomes assessed included maternal vitamin D status, metabolic parameters, neonatal outcomes, immune function, bone health, and long-term child development. The important methodological features and baseline characteristics of the included trials are summarised in **Table 1**.

Key findings by outcome domain

Maternal vitamin D status

Most studies showed a dose-dependent increase in maternal serum 25(OH) D levels, though optimal dosing varied across populations:

- Supplementation with ≥ 2,000 IU/day was consistently associated with achieving maternal sufficiency (≥ 30 ng/mL), particularly in baseline-deficient populations (*e.g.*, Motamed [35], Enkhmaa [38], Wagner [49]).
- Lower doses (< 1,000 IU/day) often failed to correct deficiency (*e.g.*, Alhomaid [23], Stoutjesdijk [57]).
- Daily doses of 4,000-5,000 IU/day were found to be the most effective in correcting severe deficiency and maintaining adequate levels throughout pregnancy (Roth [45], Hollis [26], Nausheen [69]).

Table 2 summarises the included studies' dose-response categories.

Preeclampsia

Evidence remains mixed regarding the effect of vitamin D supplementation on the incidence of preeclampsia:

- Kabuyanga *et al.* [36] reported a significant reduction in preeclampsia risk with monthly 60,000 IU vitamin D3.
- In contrast, Mirzakhani *et al.* [16] found no significant difference in preeclampsia rates between women receiving 4,400 IU/day *vs* 400 IU/day.
- Subgroup analyses suggest that achieving a 25(OH) D level > 30 ng/mL may be more important than dose alone (Mirzakhani [16], Schulz [29]).

Gestational Diabetes Mellitus (GDM)

Some trials showed beneficial effects of vitamin D on glucose metabolism, while others did not:

- Asemi *et al.* [54] found reduced polyhydramnios and neonatal hyperbilirubinemia with supplementation among GDM patients.
- Yap *et al.* [59] observed no improvement in glucose metabolism despite increased vitamin D levels.
- Mojibian *et al.* [65] found that 50,000 IU vitamin D every 2 weeks significantly reduced the incidence of GDM.
- Overall, there is limited evidence to support routine high-dose vitamin D as a preventive strategy for GDM.

Certain Maternal Illnesses (HIV, Multiple Sclerosis, Perinatal Depression)

Few trials focused on the impact of vitamin D supplementation on specific maternal conditions:

- Regarding perinatal depression, Vaziri *et al.* (2016) found that 2,000 IU/day of vitamin D3 during late pregnancy was effective in decreasing depression scores among pregnant women [39].
- For HIV, Sudfeld *et al.* (2022) found no significant effect of 3,000 IU/day of vitamin D3 on maternal disease progression or infant outcomes such as small-for-gestational age birth or stunting at one year [21].
- Concerning multiple sclerosis (MS), Etemadifar *et al.* (2015) reported that high-dose vitamin D3 supplementation (50,000 IU/week) improved disability scores and decreased relapse rates in pregnant women with MS [73].

While only a few trials addressed these conditions, the available data suggest that vitamin D supplementation may offer some benefit, particularly in populations with severe deficiency or pre-existing conditions.

Maternal bone health

One trial specifically examined the impact of vitamin D supplementation on maternal postnatal bone indices [28]. The study reported that 1,000 IU/day of vitamin D3 during pregnancy was associated with lower postpartum bone resorption markers, suggesting a beneficial effect on maternal bone metabolism.

Additionally, urinary CTX, a marker of bone resorption, was inversely related to postpartum bone mass, indicating that maintaining adequate vitamin D status during pregnancy may help preserve maternal skeletal integrity.

These findings support the hypothesis that vitamin D supplementation may contribute to maternal bone health, although more robust and long-term studies are required.

Neonatal and child outcomes

High-dose vitamin D supplementation (> 2,000 IU/day) improved neonatal vitamin D status but had inconsistent effects on clinical outcomes:

- Brustad *et al*. [64] found improved bone mineralisation at age 6 years with high-dose supplementation.
- El-Heis *et al*. [12] observed a reduced incidence of atopic eczema in the first year of life among infants whose mothers received 1,000 IU/day of vitamin D3 from 14 weeks' gestation to delivery.
- Litonjua *et al*. [37, 42] reported no significant impact on childhood asthma or wheezing.
- Evidence suggests supplementation improves cord blood vitamin D levels, but long-term benefits remain uncertain.

Immune and neurodevelopmental effects

Emerging evidence suggests potential immunomodulatory and neurodevelopmental benefits:

- Zerofsky *et al.* [43] found that 2,000 IU/day increased regulatory T cell immunity, potentially reducing inflammation-related adverse outcomes.
- Sass *et al.* [67] found no significant improvement in offspring neurodevelopment with high-dose supplementation, though earlier studies like Rodgers *et al.* [14] suggested a positive association with higher early-life 25(OH)D concentrations.

Dosing and safety

Supplementation up to 4,000-5,000 IU/day was generally safe and effective in improving maternal vitamin D status:

- No cases of hypercalcemia or toxicity were reported in trials using ≤ 5,000 IU/day (Roth [45], Hollis [26]).
- Higher bolus doses (*e.g.*, 50,000 IU every 2 weeks) also showed safety but require further study before being widely adopted (Mojibian [65], Singh [56]).

Factors influencing response to supplementation

Baseline vitamin D status significantly influenced response to supplementation:

- Women who were severely deficient at baseline often required higher doses (> 2,000 IU/day) to achieve sufficiency (Alhomaid [23], Moon [34]).
- Maternal weight gain, season of supplementation, and genetic variation (*e.g.*, DHCR7, CYP2R1, GC genes) also affected outcomes (Moon [40], Motamed [35]).

DISCUSSION

Main findings

This integrative systematic review synthesises evidence from 64 randomised controlled trials (RCTs) evaluating vitamin D supplementation during pregnancy. A rigorous search strategy was employed using high-quality databases (PubMed and Cochrane Library), ensuring comprehensive inclusion of studies conducted across diverse populations and settings.

The overall findings indicate that vitamin D supplementation during pregnancy is safe and can be recommended as part of prenatal care, particularly in populations with widespread deficiency. While improvements in maternal and neonatal vitamin D status were consistently observed, its impact on specific clinical outcomes such as preeclampsia and gestational diabetes remains inconclusive. The most consistent benefit was seen with daily doses ≥ 2,000 IU, which appear to be most effective in correcting maternal deficiency and improving neonatal vitamin D levels.

Strengths and limitations

This review builds on extensive literature and includes a large number of RCTs published over the past decade. Its strength lies in the thematic organisation of findings, which provides practical guidance for clinicians and researchers despite the heterogeneity in trial design.

By grouping studies based on outcome domains (*e.g.*, maternal vitamin D status, metabolic effects, rare comorbidities), we were able to identify patterns in dosing efficacy and safety. This thematic synthesis enhances interpretability and supports decision-making in clinical practice.

However, significant variation across studies, in terms of dosing regimens, baseline vitamin D status, timing of supplementation, and outcome definitions, limited our ability to perform a meta-analysis. These differences underscore the need for more standardised reporting in future trials.

Additionally, many included studies lacked detailed data on adverse events or long-term child development, limiting conclusions regarding extended benefits or safety beyond pregnancy.

Interpretation and comparison with existing literature

Regarding the safe dose of vitamin D during pregnancy, global recommendations vary widely, from 200 to 4,000 IU/day. In line with recent evidence, our review confirms that supplementation up to 4,000 IU/day is generally safe, with no reported cases of hypercalcemia or toxicity across multiple trials Roth [45], Hollis [26], Nausheen [69]. Some authors have even suggested that higher daily doses up to 10,000 IU/day may be well tolerated in deficient populations, though further research is needed before such recommendations can be made broadly [1, 5].

The fat-soluble nature of vitamin D supports the potential use of intermittent dosing strategies, such as weekly or monthly boluses. Roth *et al*. [44-46] demonstrated the efficacy and safety of high-dose intermittent regimens in raising maternal and neonatal vitamin D concentrations. Such approaches may be particularly beneficial in low-resource settings where daily adherence is challenging. However, these regimens require further long-term evaluation to ensure comparable clinical benefits and avoid potential risks associated with fluctuating serum levels.

In addition to the commonly reported outcomes, emerging evidence suggests that adequate vitamin D status during pregnancy may reduce the risk of rare but clinically relevant complications, for example observational evidence links sufficient vitamin D levels with a lower risk of preterm birth and first-trimester abortion [74].

Case reports highlight associations between severe maternal vitamin D deficiency and unusual conditions such as neonatal craniotabes and pregnancy-associated transient osteoporosis of the hip [75, 76].

These findings emphasise the importance of identifying and addressing profound vitamin D deficiency, particularly in high-risk groups such as women with limited sun exposure, darker skin pigmentation, or poor dietary intake.

Despite compelling evidence of vitamin D's role in perinatal health, there remains a need for well-designed RCTs to establish optimal dosing strategies and clarify its impact on major pregnancy complications. Future trials should focus on populations with a high prevalence of vitamin D deficiency and incorporate subgroup analyses based on baseline status, genetic factors, and timing of supplementation.

Furthermore, while this review focuses on vitamin D supplementation, it is important to reinforce that dietary improvement and lifestyle modifications remain essential components of prenatal care. Supplementation becomes particularly critical during pregnancy when physiological demands increase and circulating vitamin levels often decline without intervention.

CONCLUSIONS

Despite inherent limitations that constrain definitive conclusions, several important findings emerged from this systematic review. Our analysis incorporates data from numerous trials not previously included in existing reviews, offering updated insights into the role of vitamin D supplementation during pregnancy. High-quality intervention stu-

dies support the beneficial effects of vitamin D on maternal, foetal, and early childhood health outcomes. Supplementation with 4,000 IU/day appears safe and most effective in improving maternal and neonatal 25(OH) D concentrations.
However, optimal dosing should consider individual factors such as baseline vitamin D status, ethnicity, gestational timing, and season of supplementation. These variables significantly influence the response to vitamin D supplementation and should guide clinical decision-making.
We hope these findings contribute to ongoing discussions and stimulate further research aimed at refining recommendations for vitamin D use in pregnancy, ultimately leading to improved maternal and child health outcomes.

COMPLIANCE WITH ETHICAL STANDARDS

Authors' contribution
B.H.A-I.: Conceptualization, data curation, formal analysis, writing – original draft. I.T.: Visualization, writing – review & editing.

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Intrauterine device extrauterine dislocation in women with previous caesarean section: two case reports and literature review

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ABSTRACT

Background. Intrauterine contraceptive devices (IUCDs) are one of the most frequently chosen methods by patients after counselling, especially following a delivery (vaginal or caesarean) or a voluntary abortion. Although displacement of the IUCD into the lower uterine segment or cervix is common (approximately 10.4%), uterine perforation occurs in about 0.1% of cases. Meanwhile, the rate of caesarean sections (CS) has been steadily increasing, and literature reports a higher risk of uterine perforation when the device is placed within the first 4-8 weeks after surgical delivery, with perforation rates plateauing around 22-23 weeks after postpartum insertion.

Case presentation. We describe two similar cases of extrauterine dislocated copper IUCDs in patients with a single previous pregnancy, which ended in a CS more than two years earlier. In both cases, ultrasound examination clearly revealed IUCD displacement. Both patients underwent uncomplicated laparoscopic removal of the device.

Conclusions. Persistent pelvic pain in IUCD users should be thoroughly investigated through clinical and imaging analysis. Diagnosis of IUCD displacement may require urgent or emergent management.

INTRODUCTION

Intrauterine contraceptive device (IUCD) is one of the most commonly used methods of long-acting reversible contraception (LARC) due to its high efficacy, safety [1], user-friendliness, and patient satisfaction [2]. An increasing trend in LARC use has been reported compared to other contraceptive methods in recent years [3]. Comprehensive and transparent information about this method appears to improve compliance and consideration [4].
Major complications associated with IUCD use include extrauterine dislocation, perforation, expulsion, and ectopic pregnancy [5]. Although IUCD expulsion within an intact uterus (cervix or lower uterine segment) is common (approximately 10%),

uterine perforation is rare, with a reported incidence of 1.6 cases per 1,000 insertions [6]. Perforation may be diagnosed early or remain asymptomatic and undetected for several years. The risk of perforation is higher when the IUCD is positioned within 4-8 weeks after delivery or elective abortion [7]. Similarly, there is an increased risk of uterine perforation during the first years after insertion, with a plateau around 22-24 weeks [8].
There are very few studies on the risk of uterine perforation after IUCD placement in women who had a caesarean sections (CS) more than 24 months earlier and this is a major research gap. An epidemiological analysis indicates a significant increase in risk associated with CS and multiple uterine surgeries [9-10]. This is relevant for the large number of women worldwide who have undergone a caesarean section, as well as for the numerous studies exploring the necessity of placing an IUCD at the time of caesarean delivery or LARCs in the post-partum period to prevent unintended pregnancies [11].
Diagnosis and localization of an extrauterine dislocated IUCD are performed using ultrasound, plain abdominal X-ray, or both. Levonorgestrel-releasing IUCDs are more likely than copper IUCDs to show discrepancies between presurgical evaluation and actual position after extraction [12].

CASE PRESENTATION

Here, we present two similar cases of copper IUCD displacement, combining them with a comprehensive literature review. Both patients chose non-hormonal reversible contraception with a copper IUCD, without any apparent difficulty. An intravaginal ultrasound examination confirmed the device's proper placement.
Neither patient had any comorbidities. However, they shared a history of a single previous pregnancy that ended in a CS more than two years earlier. Both presented to our emergency room about two months after IUCD insertion, complaining of abnormal uterine bleeding and vague pelvic pain.
The clinical exam began with a speculum examination, which showed no signs of atypical cervicovaginal discharge or IUCD strings visible outside the external cervical os. A subsequent bimanual examination revealed an anteflexed uterus, regular in shape and freely movable, with mild parametrial tenderness but no palpable masses.
Routine blood tests were within normal limits. Ultrasound examination clearly showed IUCD displacement: in the first case, the device was embedded in the right parametrium (**Figure 1**); in the second case, the device was lodged in the Douglas pouch [3]. No free fluid or haematoma was detected. Both patients were afebrile and asymptomatic for urinary or intestinal disorders. A plain abdominal X-ray confirmed IUCD displacement.
Both patients underwent laparoscopic removal of the device. In both cases, a 12 mm optic trocar was placed, and pneumoperitoneum was achieved using the open approach. Subsequently, two 5 mm ancillary trocars were placed in the iliac fossae. No intra-abdominal adhesions or visceral lesions were found during surgery, and the uterus appeared intact. The device was removed with an atraumatic grasper. The postoperative course was uneventful, and both patients were discharged the following day.
At follow-up, both patients requested oral contraception.

DISCUSSION

In today's healthcare landscape, the proliferation of innovative contraceptive methods and devices provides a wide range of options for patients and gynaecologists. Unfortunately, the introduction of these new instruments may lead to uncommon complications, some of which have already been described in the literature [13].
The IUCD is an increasingly popular and highly effective contraceptive method, with an efficacy rate exceeding 99% [14]. The primary complications

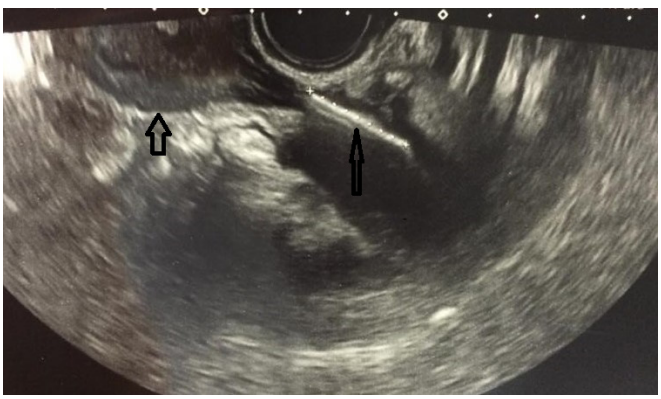

Figure 1. *Transvaginal ultrasound in transverse section showing IUCD embedded in the right parametrium.*

The two arrows indicate the uterus and the IUCD. Notice the typical hyperechoic profile of the device.

associated with these devices include intrauterine and extrauterine displacement. The latter is associated with uterine perforation. Uterine perforation events involving these devices are rare but may be more frequent in the postpartum period and during lactation.

The role of previous CS in increasing the risk of uterine perforation needs further investigation, especially given the rising CS rates. It is well known that the process of cicatrization of the myometrial wall after a caesarean is problematic, with well-known complications such as uterine wall defects, which can lead to perforation or uterine rupture in some cases [15]. Few studies have examined the risk of perforation beyond 24 months after CS, and the rarity of this event limits the available data to determine whether a previous CS constitutes a significant risk factor for complications.

The sequence of events leading to uterine perforation and IUCD migration may be influenced by uterine malposition, malformations, previous CS, multiparity, postpartum period, lactation and the operator's experience, potentially resulting in primary perforation. Regarding timing of insertion World health organization is very clear stating that IUCDs can be inserted within 48 hours after delivery [16] However, a previous CS may predispose to secondary perforation due to the increased susceptibility of the uterine wall to gradual pressure and subsequent necrosis caused by the IUCD [17].

Non-specific and vague symptoms should never be underestimated in patients with an IUCD, and prompt clinical evaluation is essential.

A plain X-ray is unsuitable for precise localization of the device, as it cannot reliably differentiate between an IUCD correctly positioned within the uterine cavity and a case of partial or complete uterine perforation. Therefore, intracavitary radiopaque contrast is required for an accurate differential diagnosis. However, in our cases, the IUCDs were unequivocally dislocated to extrauterine sites.

The diagnosis of IUCD perforation necessitates an urgent minimally invasive surgical approach, particularly in symptomatic patients. The choice of technique – hysteroscopy, cystoscopy, colonoscopy, or laparoscopy – depends on the IUCD's abnormal location [18], considering the potential risks of pathological adhesion formation, intestinal or urinary tract injury, and medico-legal implications.

Uterine perforations rarely result in severe complications such as bowel or bladder injury, sepsis, or peritonitis. For extrauterine IUCDs, laparoscopic surgery is the preferred approach; however, laparotomy is warranted in cases of bowel perforation or severe sepsis. Electrocautery should be avoided due to concerns about thermal energy transmission by the copper IUCD during surgical removal [19]. An ultrasonic scalpel (*e.g.*, Harmonic) is recommended for adhesion removal to minimize thermal energy dispersion.

Patients with IUCDs perforation exhibited a higher incidence of retroflexed uterine positions and various uterine anomalies – including septate and bicornuate uteri and fibroids – compared to controls [20]. Notably, submucosal fibroids were more prevalent among women with extrauterine dislocated IUCDs [20].

No significant difference in uterine perforation rates was observed when comparing the Levonorgestrel intrauterine system (LNG-IUS) with the copper IUCD. Most early perforations were detected due to symptoms of pain and/or bleeding. However, perforation was asymptomatic in 29% of LNG-IUS cases and 17% of copper IUCD cases.

While some patients exhibit signs and symptoms suggestive of perforation (such as pain or bleeding), many remain asymptomatic at the time of diagnosis. Patients with IUCD migration are also at risk of unintended pregnancy [21]. Symptoms vary depending on the extrauterine location of the IUCD.

Perforated IUCDs have been found in various locations, including the omentum (26.7%), pouch of Douglas (21.5%), colonic lumen due to perforation (10.4%), myometrium (7.4%), broad ligament (6.7%), free within the abdominal cavity (5.2%), small bowel serosa (4.4%), colonic serosa (3.7%), and mesentery (3%). Rare locations include the bladder, appendix, abdominal wall, fallopian tube, ovary, retroperitoneum, and small bowel with perforation.

The World Health Organization recommends prompt removal of migrated IUCDs, as chronic cases may lead to granulation tissue formation, complicating retrieval and increasing the risk of adhesions [22, 23]. Symptoms of colorectal perforation can be nonspecific. Some authors report a case of a 77-year-old woman with a positive faecal occult blood test and no symptoms, in whom two IUCDs were found located in the transverse colon [24].

IUCD bladder perforation typically manifests as recurrent and refractory cystitis, with only temporary response to treatment. Patients generally present with pyuria and a positive urine culture [25]. In

most cases, the IUCD is freely floating in the abdominal or pelvic cavity, often encased in adhesions, which can lead to infertility, chronic pain, and intestinal obstruction. Rarely, an intraperitoneal IUCD may perforate adjacent structures, resulting in peritonitis, fistula formation, or haemorrhage [12].

First, the patient is assessed to rule out the possibility of spontaneous expulsion. A complete physical and gynaecological examination is then performed to assess abdominal pain, uterine tenderness, and abnormal cervicovaginal discharge. A subsequent ultrasound examination helps determine whether the IUCD is intrauterine or extrauterine, as observed in our two cases [26].

Three-dimensional ultrasound (3D US) is often useful in further characterizing displacement or myometrial perforation [25]. Standard two-dimensional ultrasound (2D US), whether vaginal or abdominal, typically provides an accurate visualization of the IUCD shaft but may not always clearly identify the location of its side arms. Copper IUCDs typically appear as highly echogenic and are easier to distinguish from a medicated device, which is smaller and tends to be iso to hyperechogenic. The 3D coronal view of the uterus is particularly effective in illustrating the spatial relationship between the IUCD shaft, arms, and the endometrial cavity. Thus, the IUCD location was misidentified in 12% of cases using transvaginal ultrasound. Additionally, IUCDs were not detected in 9% of patients undergoing office-based 2D ultrasound [26]. Embedment occurs when the IUCD penetrates the endometrium or myometrium without extending through the serosa. Ultrasound is the preferred initial imaging modality for suspected IUCD perforation. Complete perforation is a more severe complication, occurring when the IUCD penetrates all three layers of the uterus, extending partially or completely into the extraperitoneal tissues, as in our first case, or into the peritoneal cavity, as in our second case.

In cases of suspected major surgical complications related to intra-abdominal IUCDs, such as visceral perforation, abscess formation, or bowel obstruction, CT is considered the gold standard imaging modality [25, 27].

To prevent this complication, we propose a management strategy aimed at reducing the risk, particularly in cases of isthmocele (also referred to as caesarean scar defect, niche, or diverticulum), which is becoming increasingly prevalent in gynaecological practice (**Figure 2**). It is crucial to highlight the major

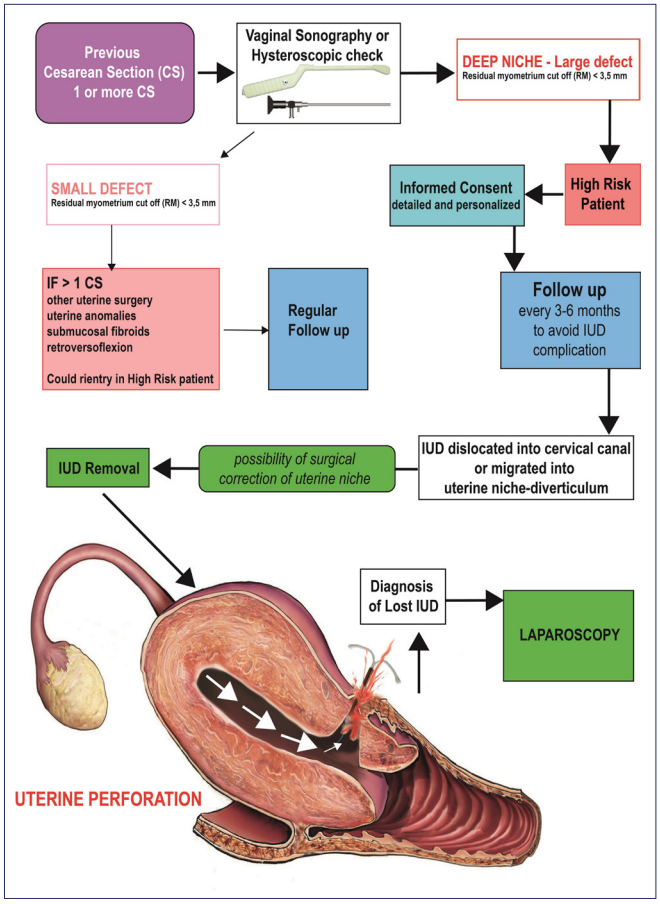

Figure 2. *Proposal management and mechanism of dislocated IUCD in women with previous cesarean section.*
CS: caesarean section.

technological advancements achieved in recent years, particularly in ultrasound and hysteroscopy.

In this context, an accurate assessment of the uterine niche is essential. An isthmocele is defined as a myometrial discontinuity or a triangular anechoic defect in the anterior uterine wall. It can be classified as a small or large defect based on the residual myometrial thickness at the site of the previous hysterotomy. Although often asymptomatic, its primary clinical manifestation is abnormal or postmenstrual bleeding, and some patients may report chronic pelvic pain.

Risk factors for uterine niche formation include a retroflexed uterus and multiple caesarean sections (CS). A residual myometrial thickness of < 3.5 mm is considered a large defect, whereas a thickness of ≥ 3.5 mm is classified as a small defect. These defects can be accurately evaluated using ultrasound and magnetic resonance imaging (MRI) [28].

In selected high-specialization centres, hysteroscopic evaluation may be performed to assess the presence of a uterine niche, providing a qualitative

measurement of the residual myometrial thickness and detecting possible perforations. Careful patient selection and strict evaluation criteria are essential, as the insertion of an intrauterine device (IUCD) in such cases may lead to severe complications that are sometimes difficult to diagnose.

Therefore, in women with a history of caesarean section, the procedure must be carefully tailored, taking into account previous uterine surgeries and any underlying anomalies. Even a seemingly simple procedure can sometimes trigger complications related to preexisting structural defects

A detailed and personalized informed consent with comprehensive counselling must be provided to women with a history of caesarean section (CS).

In the informed consent, according to recent literature, it is crucial to specify that a residual myometrial thickness of ≥ 3.5 mm is the cutoff to reduce the risk of uterine perforation or bladder injury.

A combined laparoscopic-hysteroscopic approach should be preferred in cases of significant defects. After dissecting the vesicouterine pouch, the surgeon incises the scar tissue of the niche, guided by transillumination from the hysteroscopic route, and sutures the uterine wall in multiple layers to reinforce the defect.

Due to the increasing number of CS procedures, the previously rare complications of IUCD perforation are becoming more common. There is a need for evidence-based, safe, and effective surgical strategies to manage these patients.

CONCLUSIONS

Pelvic symptoms in women using an IUCD should never be overlooked. Clinical and imaging evidence of IUCD extrauterine dislocation necessitates an urgent, minimally invasive surgical approach, particularly in symptomatic patients. This is a small case series, and further research is required to determine the safest interval after a previous CS for IUCD insertion, aiming to reduce the risk of secondary uterine perforation and subsequent device migration. Minimally invasive techniques remain the gold standard for retrieving extrauterine dislocated IUCDs. Informed consent should be thorough and tailored to the patient, ensuring the woman is informed of all potential risks. Moreover, a more rigorous follow-up schedule should be adopted to effectively monitor complications.

COMPLIANCE WITH ETHICAL STANDARDS

Authors' contribution

A.P., M.V., D.C.: Conceptualization, data curation, formal analysis, writing – original draft, writing – review & editing. A.D., A.M., M.C.: Validation, visualization, writing – original draft, writing – review & editing. G.R.D.: Methodology, project administration, supervision, writing – original draft, writing – review & editing. D.D.G.: Data curation, writing – review & editing.

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Disclosure of interests

The authors declare that they have no conflict of interests

Ethical approval

N/A.

Informed consent

Informed consent for data collection for research purpose and publication was retrieved by every patient included in the study. All personal data have been anonymized in the current publication.

Data sharing

All data are on the public online repository.

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Ultrasound features *versus* magnetic resonance imaging features for diagnosis of placenta accreta spectrum

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ABSTRACT

Objective. Prenatal diagnosis of placenta accreta spectrum (PAS) can minimize maternal morbidity and mortality. This study aims to compare the accuracy of trans-abdominal ultrasound with colour and 3D power Doppler *versus* magnetic resonance imaging (MRI) to diagnose PAS.

Materials and Methods. This study included seventy-five pregnant women with placenta previa. They underwent grey-scale trans-abdominal ultrasound with colour and 3D power Doppler and non-contrast MRI to confirm placenta previa and detect any suggestive features of placental invasion. All enrolled patients were divided into two groups based on the intraoperative assessment of placental adherence. Group 1 included cases with adherent placenta previa (n = 55), and Group 2 included non-adherent placenta previa cases (n = 20).

Results. The best ultrasonographic feature to diagnose PAS was the presence of intraplacental lacunae with turbulent blood flow by colour Doppler, with a sensitivity of 96.36%, specificity of 100%, positive predictive value of 100%, negative predictive value of 90.91%, values, and accuracy of 97.33%. The best MRI feature to diagnose PAS was the presence of dark intraplacental bands on T2-weighted images, with a sensitivity of 92.73%, specificity of 80%, positive predictive value of 92.73%, negative predictive value of 80%, and accuracy of 89.33%. However, inter-observer and intra-observer reliability were not evaluated.

Conclusions. Both ultrasound with Doppler and MRI offer high sensitivity, specificity, positive and negative predictive values, and accuracy for diagnosing PAS in high-risk women. We suggest using MRI when ultrasound is inconclusive.

INTRODUCTION

Placenta previa is an obstetric disorder with a prevalence of 52 per 10,000 pregnancy cases, in which the placental tissue partially or fully extends over the internal cervical orifice [1, 2]. It is associated with adverse maternal and foetal outcomes, including placental adhesion to the uterine wall, which

is now known as the "placenta accreta spectrum" (PAS) [3].

PAS is described as an aberrant trophoblastic invasion of a portion or all placenta into the uterine myometrium. Histopathologically, it is classified into three grades: placenta accreta (chorionic villi in touch with myometrium), placenta increta (chorionic villi infiltrating the myometrium), and placenta percreta (chorionic villi piercing the myometrium and uterine serosa and reaching the urinary bladder wall) [4].

PAS is the most common cause of "difficult" caesarean sections due to improper placental separation. Caesarean hysterectomy has been one of the options to avoid massive bleeding [5].

This condition increased maternal mortality by 7% and increased surgical morbidities such as massive blood transfusion, infection, urological injuries, fistula formation, and ICU admission [6, 7]. Prenatal diagnosis of PAS provides vital benefits, including planning to arrange all management modalities. Furthermore, high-risk instances, such as bladder involvement and cervical invasion, could be recognized, as well as massive blood loss could be avoided [8, 9].

Gray-scale ultrasound has been widely used for evaluating placental position and implantation, and it is regarded as a basis for diagnosing PAS due to its widespread availability and good diagnostic accuracy [10]. Doppler ultrasonography with 3D option could be used as a supplemental tool for the prenatal diagnosis or exclusion of PAS [11, 12].

Magnetic Resonance Imaging (MRI) relies significantly on the radiologist's skills and competence when evaluating the images [13]. When ultrasound results are questionable and unclear, or if the placenta is positioned posteriorly, MRI is advised as a supplementary imaging tool. In addition, MRI is probably used to arrange the caesarean section and/or peripartum hysterectomy when diagnosis is established by the ultrasound [14]. However, MRI can accurately identify the increta and percreta kinds of PAS, whereas the accreta type remains challenging to identify [15].

This study aims to compare the efficiency of grey-scale ultrasound with colour and 3D power Doppler *versus* MRI in diagnosing PAS and evaluate the sensitivity, specificity, positive and negative predictive values, and accuracy of each ultrasonographic and MRI feature.

MATERIALS AND METHODS

Study registration, ethical and methodological standards

This study included 75 pregnant women with placenta previa who were admitted to the Obstetrics and Gynecology Department at Kasr Al Ainy Hospital, Cairo University, with suspected placental invasion and/ or adherence by outpatient clinic 2D ultrasound. The research protocol was approved by the Research Ethics Committee. All methods were conducted following relevant guidelines and regulations.

Patient and public involvement

Our inclusion criteria were age between 18 and 40 years, BMI less than 35 kg/m^2, and singleton pregnancy at 34-37 weeks of gestation in whom placenta previa was implanted over previous caesarean scar. We excluded cases of gestational trophoblastic disease or other placental tumours, placental anomalies, uterine tumours (*e.g.*, fibroids), uncontrolled medical disorders, presence of contraindication to MRI, or cases in active labour on admission.

Data collection

After signing an informed written consent form, all cases were subjected to a history taking and general and obstetric examination. They also underwent grey-scale trans-abdominal ultrasound with colour and 3D power Doppler ultrasound and non-contrast MRI to confirm placenta previa and detect any suggestive features of placental invasion. Ultrasound and MRI for all cases were done by a senior obstetrician and radiologist, respectively, who was experienced in ultrasonographic and MRI assessment of placental invasion. Both were uninformed of each other's results to avoid bias.

Suggestive findings of placental invasion by grey scale ultrasound included loss of retroplacental sonolucent zone, loss of hyperechogenic uterine serosal bladder interface, irregular bladder wall, uterine bulging, exophytic uterine masses inside the bladder, and abnormal placental lacunae. Suggestive findings of placental invasion by Doppler ultrasound included lacunar flow pattern, sonolucent vascular areas with turbulent flow in the form of high-velocity (PSV > 15 cm/s) and low-resistance waveform, hypervascularity of the uterine-bladder interface with aberrant bridging vessels between placenta and bladder, and remarkably dilated vessels over the peripheral subplacental area.

Features suggestive of placental invasion by MRI included heterogeneous signal intensity within the placenta, dark intraplacental bands on T2-weighted images, focal thinning or absence of the myometrium at the site of placental implantation, bladder tenting as a sign of possible bladder invasion, and an outer uterine wall bulge caused by the placenta. Surgical intervention was done for all cases. After separating both rectus muscles and dissecting the parietal peritoneum, an intra-operative assessment of the placenta by the surgeon was done to reveal the condition of placenta previa regarding adherence, degree of placental adherence (accreta, increta, or percreta) and degree of myometrial invasion in case of adherent placenta (focal, partial, or total invasion). Proper dissection and mobilization of the bladder were done. If a bladder invasion or injury occurred, it was reported. All operative procedures (*e.g.*, caesarean hysterectomy, conservative management, internal iliac arteries ligation, Bakry ballon application), blood transfusion, and foetal or maternal mortalities were reported. In the case of caesarean hysterectomy, a pathological examination of the uterine specimen was done, as it is considered the gold standard to diagnose PAS and its degree of invasion.

Sample size

The sample size was -calculated to be 75 patients according to this equation: $n = [(Z\ alpha/2)/E] \times [Sn\ (1\text{-}Sn)/P]$, where Z alpha/2 = 1.96, E = The margin of error, P = proportion of PAS among abnormally implanted placentae, and Sn = Sensitivity of grey-scale ultrasound in diagnosing PAS.

Statistical analysis

The "Statistical Package for the Social Sciences" (SPSS) version 26 (IBM Corp., Armonk, NY, USA) was used to code and input the data. To summarize data, quantitative data was presented in the form of mean, standard deviation, median, minimum, and maximum, while categorical data was presented in the form of frequency and percentage. The non-parametric Mann-Whitney test was employed for quantitative variable comparisons, while Chi-square analysis was done to compare categorical data. An exact test was applied when the expected frequency was less than 5. The standard diagnostic indices of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated. P-values lower than 0.05 were considered significant.

RESULTS

Seventy-five pregnant women with placenta previa were recruited according to the eligibility criteria. All enrolled patients were assigned into two groups based on the intraoperative assessment of placental adherence. Group 1 included women with adherent placenta previa or PAS (55 cases), either placenta accreta (n = 33), increta (n = 12), or percreta (n = 10). On the other hand, Group 2 included women with non-adherent placenta previa (20 cases).

The age, the body mass index (BMI), and the obstetric history are demonstrated in **Table 1**. There was no statistical significance difference between groups on age (p = 0.141), BMI (p = 0.155), gravity (p = 0.516), parity (p = 0.561), and the number of previous caesarean deliveries (p = 0. 798).

Table 2 shows the diagnostic indices for each ultrasonographic feature to predict PAS. The best ultrasonographic feature to diagnose PAS was the presence of intraplacental lacunae with turbulent blood flow by colour Doppler, with a sensitivity of 96.36%, specificity of 100%, positive predictive value of 100%, negative predictive value of 90.91%, values, and accuracy of 97.33%.

Table 3 shows the diagnostic indices for each MRI feature to predict PAS. The best MRI feature to diagnose PAS was the presence of dark intraplacental bands on T2-weighted images, with a sensitivity of 92.73%, specificity of 80%, positive predictive value of 92.73%, negative predictive value of 80%, and accuracy of 89.33%.

Operative data among the study groups are shown in **Table 4**. Conservative management had significantly succeeded in women with non-adherent placenta previa (95% of cases), compared to cases

Table 1. *Demographic data of both groups.*

	Group 1 (PAS) (n = 55)	Group 2 (Non-adherent PP) (n = 20)	P-value
Age (years)	30.25 ± 4.99 30 (23 - 39)	31.9 ± 5.32 33.5 (24 - 38)	0.141
BMI (kg/m²)	28.79 ± 2.83 29.6 (24.7 - 34.3)	29.71 ± 3.06 30.7 (25 - 33.8)	*0.155*
Gravity	3.49 ± 2.24 3 (0 - 7)	3.9 ± 2.27 4 (0 - 7)	0.516
Parity	2.91 ± 2.13 3 (0 - 7)	3.25 ± 2.2 3 (0 - 7)	0.561
Number of previous caesarean deliveries	2.22 ± 1.73 2 (0 - 7)	2.4 ± 1.98 2 (0 - 7)	0.798

Table 2. *Ultrasonographic features to diagnose PAS.*

		Group 1 (PAS) (n = 55)	Group 2 (Non-adherent) (n = 20	P-value	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Accuracy
Intraplacental lacunae and turbulent blood flow by colour Doppler	Positive	53 (96.40%)	0 (0%)	< 0.001	96.36%	100%	100%	90.91%	97.33%
	Negative	2 (3.60%)	20 (100%)						
Increased vascularity by colour and 3D power Doppler	Positive	32 (58.20%)	5 (25%)	0.011	58.18%	75%	86.49%	39.47%	62.67%
	Negative	23 (41.80%)	15 (75%)						
Interrupted bladder uterine interface	Positive	42 (76.40%)	3 (15%)	< 0.001	76.36%	85%	93.33%	56.67%	78.67%
	Negative	13 (23.60%)	17 (85%)						
Bulging uterine lobe inside the bladder	Positive	37 (67.30%)	0 (0%)	< 0.001	67.27%	100%	100%	52.63%	76%
	Negative	18 (32.70%)	20 (100%)						
At least one suggestive feature by ultrasound	Positive	54 (98.20%)	5 (25%)	< 0.001	98.18%	75%	91.53%	93.75%	92%
	Negative	1 (1.80%)	15 (75%)						

Table 3. *MRI features to diagnose PAS.*

		Group 1 (PAS) (n = 55)	Group 2 (Non-adherent) (n = 20	P-value	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Accuracy
Outer uterine bulge by the placenta	Positive	45 (81.80%)	4 (20%)	< 0.001	81.82%	80%	91.84%	61.54%	81.33%
	Negative	10 (18.20%)	16 (80%)						
Heterogeneous signal intensity within the placenta	Positive	39 (70.90%)	5 (25%)	< 0.001	70.91%	75%	88.64%	48.39%	72%
	Negative	16 (29.10%)	15 (75%)						
Dark intraplacental bands on T2-weighted images	Positive	51 (92.70%)	4 (20%)	< 0.001	92.73%	80%	92.73%	80%	89.33%
	Negative	4 (7.30%)	16 (80%)						
Bladder tenting (possible bladder invasion)	Positive	13 (23.60%)	0 (0%)	0.015	23.64%	100%	100%	32.26%	44%
	Negative	42 (76.40%)	20 (100%)						
Focal thinning or myometrial gaps	Positive	42 (76.40%)	4 (20%)	< 0.001	76.36%	80%	91.30%	55.17%	77.33%
	Negative	13 (23.60%)	16 (80%)						
At least one suggestive feature by MRI	Positive	53 (96.40%)	8 (40%)	< 0.001	96.36%	60%	86.89%	85.71%	86.67%
	Negative	2 (3.60%)	12 (60%)						

Table 4. *Operative data among the study groups.*

	Group 1 (PAS) (n = 55)				Group 2 (Non-adherent placenta previa) (n = 20)	P-value (Group 1 vs Group 2)
	Accreta (n = 33)	Increta (n = 12)	Percreta (n = 10)	All PAS cases (n = 55)		
Conservative management	3 (9.10%)	2 (16.70%)	0 (0%)	5 (9.09%)	19 (95%)	**<0.001***
Radical hysterectomy	30 (90.91%)	10 (83.33%)	10 (100%)	50 (90.91%)	1 (5%)	**<0.001***
Bilateral internal iliac artery ligation	0 (0%)	2 (16.67%)	3 (30%)	5 (9.09%)	0 (0%)	0.316
Bakry balloon tamponade	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (5%)	0.267
Bladder injury and repair	0 (0%)	1 (8.33%)	4 (40%)	5 (9.09%)	0 (0%)	0.316
Postoperative ICU admission	0 (0%)	2 (16.67%)	2 (20%)	4 (7.27%)	0 (0%)	0.568
Maternal or foetal mortality	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	NA
Number of blood units transferred	0.58 ± 0.87 0 (0 - 3)	1.08 ± 1.08 1 (0 - 3)	1.2 ± 1.39 1 (0 - 4)	0.80 ± 1.04 0 (0 - 4)	0.65 ± 1.04 0 (0 - 4)	0.521

with PAS ($p < 0.001$). On the other hand, most cases with PAS required radical hysterectomy (90.91% of placenta accreta cases, 83.33% of placenta increta cases, and 100% of placenta percreta cases), while only one case with non-adherent placenta previa ended with radical hysterectomy ($p < 0.001$). No cases with non-adherent placenta previa had bladder injury or needed postoperative ICU admission, while one case with placenta increta, and 4 cases with placenta percreta had bladder injury, and 2 cases with placenta increta and 2 cases with placenta percreta needed postoperative ICU admission. Regarding the number of blood units transferred, both groups had no significant difference ($p = 0.521$).

DISCUSSION

Main findings

PAS develops when the chorionic villi aberrantly penetrate the uterine wall. Placenta previa, recurrent caesarean deliveries, multiparity, previous abortion, and previous curettage are all risk factors for PAS. Therefore, the frequency of PAS has become higher in correlation with the rising trend of caesarean deliveries. Unfortunately, this life-threatening problem may be first discovered during delivery, necessitating urgent management with an increased risk of morbidity [16]. Therefore, early diagnosis would enable a planned strategy with the potential for therapy under better-regulated circumstances and would help lessen the blood loss linked to PAS after birth. Diagnostic challenges most likely explain the present considerable difference in the reported prevalence of PAS, which ranges between 0.5% and 0.05% of pregnancies [17].

In this study, patients underwent grey-scale trans-abdominal ultrasound with colour and 3D power Doppler ultrasound and non-contrast MRI to confirm placenta previa and detect any suggestive features of placental invasion. Fifty patients out of the adherent placenta previa group ($n = 55$) underwent caesarean hysterectomy, while the remaining five patients had conservative surgery, and only one case out of 20 cases of non-adherent placenta previa underwent caesarean hysterectomy due to severe postpartum haemorrhage. We found that the best ultrasonographic feature to diagnose PAS was the presence of intraplacental lacunae with turbulent blood flow by colour Doppler, while the best MRI feature to diagnose PAS was the presence of dark intraplacental bands on T2-weighted images. MRI may be useful as a complementary technique with ultrasound (grey-scale, colour Doppler, and 3D power Doppler) for the antenatal diagnosis of placenta accreta, but it cannot replace ultrasonography as a screening test for the diagnosis of placenta accreta. MRI can be used to confirm the diagnosis of placenta accreta further.

Interpretation and comparison with other literature

Several studies were conducted to compare the efficiency of ultrasound and MRI in early diagnosis of cases with PAS [18-21]. Despite the variety of these studies and their results, MRI is reported as a non-essential tool in the diagnosis of PAS but effective in circumstances when ultrasonography is inconclusive. Maher *et al*. (2013) reviewed 577 women who had a diagnosis of placenta previa in Saudi Arabia and examined them by both ultrasound and MRI for detection of possible placental invasion. This study showed that ultrasound had higher sensitivity and specificity than MRI (95.1% and 95.5% *vs* 85.7% and 76.9%, respectively) [22].

D'Antonio *et al*. (2013) conducted a meta-analysis that included 3,707 patients from 23 studies to define the efficiency of ultrasonography in diagnosing PAS in high-risk individuals. They found that in women with low anterior placenta and prior uterine surgery, a third-trimester ultrasound has higher sensitivity and specificity in diagnosing PAS [10]. After that, they conducted another meta-analysis that included 1010 patients from 18 [13]. They conclude that the accuracy of MRI in diagnosing PAS is nearly equivalent to ultrasonography. However, MRI could be conducted when ultrasonography is inconclusive or when the placenta is positioned laterally. In addition, MRI can clarify the topography of placental invasion needed for surgical planning.

Riteau *et al.* (2014) retrospectively reviewed the medical records of 42 pregnant women whom both ultrasonography and MRI had investigated. They found that sensitivity in diagnosing PAS with ultrasound was higher than MRI (100% *vs* 76.9%). They also found that the sign of the highest sensitivity in ultrasound diagnosis was the presence of intraplacental lacunae. However, they found that the specificity with ultrasonography was lower than MRI (37.5 *vs* 50%). They also concluded that ultrasound is the basis of screening for PAS and that MRI can be supplementary to ultrasonography, especially when there are minimal ultrasound features [23].

Satija *et al*. (2015) conducted a study on 30 pregnant women with high risk of PAS. A prenatal screening was made based on both colour Doppler ultrasound and MRI. They concluded that coloured Doppler ultrasound had higher sensitivity and specificity than MRI (87.5% and 86.4% *vs* 75% and 77.3%, respectively). Although both ultrasound and MRI still have relatively good sensitivity for prenatal screening of PAS, colour Doppler ultrasonography is still the method of choice for diagnosis, while MRI is saved for situations where ultrasound is not definitive [24].

Maged *et al*. (2017) conducted a study on 100 pregnant women with placenta previa at the Obstetrics and Gynecology Department of Kasr Al-Ainy Hospital to detect the diagnostic accuracy of Doppler ultrasound in diagnosing PAS. Abdominal Doppler ultrasound findings showed a significant difference between accreta and non-accreta groups with high sensitivity of 93.65% for intraplacental lacunae, 87.3% for loss of retroplacental clear zone, 82.54% for dilated blood vessels over peripheral subplacental area, and 47.62% for hypervascularity in the uterine bladder interface [25].

Ayati *et al*. (2017) conducted a study on 82 pregnant women suspected of PAS who underwent colour Doppler ultrasonography and MRI. They found that Doppler ultrasound had higher sensitivity than MRI (87% *vs* 76%) but lower specificity (63% *vs* 83%). The study recommended that Doppler ultrasound should be done first on high-risk women for PAS. MRI can be done when Doppler ultrasound results are inconclusive for PAS because of the high specificity of MRI [18].

El-Assaly (2020) conducted a study that included 50 pregnant women with placenta previa with clinical and ultrasonographic criteria suspecting placental invasion. The study aimed to detect the diagnostic accuracy of MRI in diagnosing PAS. It showed that MRI offered a superior choice in diagnosing PAS with a sensitivity of 72% and specificity of 100% [26]. This could be attributed to the sonographer's deficient experience. Therefore, a standardized scoring system and improved sonographer training will eliminate as many diagnostic uncertainties as feasible in ultrasound results.

Barzilay *et al*. (2020) conducted a study on 28 pregnant women at high risk for PAS in the third trimester. All women underwent ultrasonographic assessment and MRI for PAS screening, and then the diagnosis was confirmed during surgery. Compared to MRI, ultrasound was found to be more sensitive (96% *vs* 83%) and more specific (60% *vs* 40%). Thus, ultrasonography is superior to MRI in diagnosing PAS [21].

Califano *et al*. (2024) conducted a study on 81 pregnant women with placenta previa who had previous caesarean section. They found that 12 cases (14.8%) had placenta lacunae, 16 cases (19.8%) had a loss of clear space, 20 cases (24.7%) had increased vascularity between myometrium and placenta, 9 cases (11.1%) had an intracervical lake, 14 cases (17.3%) had rail sign, 14 cases (17.3%) had uterovesical hypervascularity, 5 cases (6.2%) had increased vascularity in the most lower part of lower uterine segment, 8 cases (9.9%) had disrupted bladder-myometrial interface. Cases of bladder-myometrial interface disrupted on ultrasonography had a 73-fold higher incidence of placenta accreta than those without. They concluded that the disrupted bladder-myometrial interface was the most sensitive sign for detecting placenta accreta [27].

Strength points and limitations

Our study's principal weakness point is its small sample size. In addition, inter-observer and intra-observer reliability were not evaluated. Larger population size studies are advised, with additional inclusion criteria, to achieve more useful results.

CONCLUSIONS

Both ultrasound with Doppler and MRI offer high sensitivity, specificity, positive and negative predictive values, and accuracy for diagnosing PAS in high-risk women. The decision between these modalities should depend on equipment availability and the center's competence. Our study suggests using ultrasound assessment to screen PAS cases and preserving the use of MRI when ultrasound results are inconclusive for surgical planning, as MRI is more expensive. Future research may suggest the method of choice for each PAS type.

COMPLIANCE WITH ETHICAL STANDARDS

Authors' contributions

M.A.A., A.A.G.: Conceptualization, methodology, project administration, resources, supervision, writing - review & editing. S.O.A., M.E., S.F.D.: Data curation, formal analysis, investigation, validation, writing - original draft, writing - review & editing.

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Study registration
N/A.

Disclosure of interests
The authors declare that they have no conflict of interests.

Ethical approval
The study protocol was approved by the Research Ethics Committee of Faculty of Medicine, Cairo University, with reference number (MD-180-2019). All methods were conducted following relevant guidelines and regulations.

Informed consent
All participants gave their consent after being informed of the study's goal and design. They were given the choice to leave study at any time.

Data sharing
Data are available under reasonable request to the corresponding author upon request and with the permission of Kasr El-Ainy Hospital.

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Menstrual blood VEGF, IL-6, TGF and nerve fibre as markers of adenomyosis: a literature review

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ABSTRACT

Background. Adenomyosis is a benign uterine disease characterized by the presence of endometrial glands and stroma in the myometrium. It lacks classic physical or laboratory examination findings, which hinders clinical diagnosis. Although unlikely to replace hysterectomy, transvaginal ultrasound, or MRI, menstrual blood based-biomarker testing is expected to aid in early adenomyosis detection and consequently earlier initiation of clinical management strategies.

Objective. This literature review aims to review and summarize the expression of VEGF, IL-6, TGF, and menstrual blood nerve fibre as biomarkers of adenomyosis.

Methods. We searched for literature using four database sources, published in English within the last 10 years using the following keywords: "adenomyosis" AND "VEGF" AND "IL-6" AND "TGF" AND "Nerve fiber". Data was extracted independently by the authors and then selected based on specified inclusion and exclusion criteria.

Results. Expression of VEGF, IL-6, TGF, and endomyometrial nerve fibres in patients with adenomyosis were significantly increased in patients with adenomyosis.

Conclusions. Most research results point to the expression of VEGF, IL-6, TGF, and endomyometrial nerve fibres as potential biomarkers for adenomyosis. However, future research with better methodology still needs to be conducted before routine clinical implementation.

BACKGROUND

Adenomyosis is a benign uterine disease marked by endometrial glands and stroma embedded in the myometrium, which surrounded by smooth muscle hyperplasia [1, 2]. The true prevalence of adenomyosis is unknown, as a definitive diagnosis requires histopathological examination via hysterectomy. Current estimates of prevalence range from 8.8-61.5% in patients undergoing consecutive hysterectomies over the past 50 years. Another study conducted involving 985 women undergoing

transvaginal ultrasound in the UK found that the prevalence of adenomyosis was 20.9%. It is also reported to coexist in a number of other gynaecological conditions: leiomyoma, pelvic organ prolapse, and abnormal uterine bleeding Differences in histopathologic criteria for diagnosis, different numbers of histologic tissue samples for each hysterectomy, and providers' level of awareness contribute to this broad estimation [1].

Diagnosis of adenomyosis typically begins with clinical suspicion and is confirmed through transvaginal ultrasound and pelvic MRI. Around one-third of patients with adenomyosis are asymptomatic, while others may experience heavy menstrual bleeding (most common symptom), infertility, or pelvic pain. It also lacks any classic physical examination findings or laboratory studies that would identify it as a possible diagnosis. Furthermore, sonographic assessment of adenomyosis is hindered by low reproducibility [3, 4]. Therefore, the diagnosis of adenomyosis can be challenging and ambiguous as it requires a combination of clinical evaluation, imaging, and histopathological examination, much like other uterine conditions [5-7]. Meanwhile, prompt diagnosis of adenomyosis is critical as delays may result in disease progression, increased morbidity, and impaired fertility.

Menstrual blood based-biomarker testing could enable earlier detection of adenomyosis compared to current diagnostic methods, enabling prompt initiation of clinical management strategies and fertility treatments. While unlikely to replace hysterectomy as the gold standard or imaging tools such as transvaginal ultrasound and MRI, biomarkers could still be used as adjunct in clinical decision-making [5, 8, 9]. With an understanding of molecular and clinical pathogenesis of adenomyosis, numerous potential biomarkers can be used to detect adenomyosis. This narrative review aims to examine the current evidence four adenomyosis biomarkers that have great potential which are not yet used routinely in clinical practice, namely VEGF, IL-6, TGF, and endometrial nerve fibres. These biomarkers are expected to be applied in the future in clinical practice.

MATERIALS AND METHODS

We searched the literature using four database sources: PubMed, Cochrane, Medline, and ScienceDirect that published in English between 2014 and 2024 using the following keywords: "adenomyosis" AND "VEGF" AND "IL-6" AND "TGF" AND "Nerve fiber". Additionally, snowballing and hand searching were also done. The authors independently extracted data, starting with an initial screening of titles and abstracts (**Figure 1**). If the eligibility of an article could not be determined from the title and abstract alone, they reviewed the full text to make a final assessment. The studies obtained were then selected based on the specified inclusion and exclusion criteria. The inclusion criteria of our studies were: 1) Studies investigating the basic and clinical molecular pathogenesis of adenomyosis use human specimens, 2) Expression VEGF, IL6, TGF, and endometrium blood nerve in adenomyosis. The exclusion criteria for this study include inaccessible full text forms, narrative text, or research design. Study selection and data extraction was done by all authors, with discrepancies being resolved through discussion.

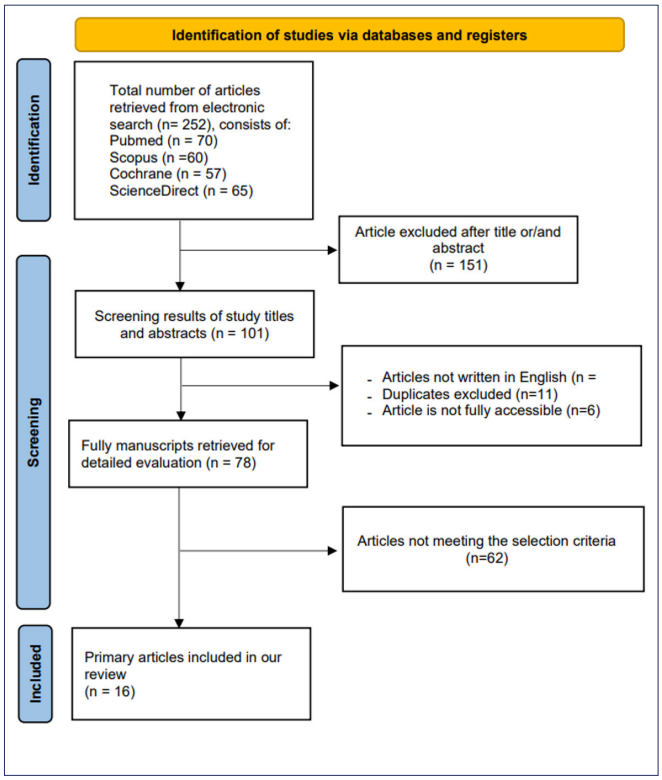

Figure 1. *Study selection process.*

RESULTS

A total of 16 articles met the research criteria and were used in this literature review study as the main findings, consisting of 6 articles discussing VEGF (**Table 1**), 3 articles discussing IL-6 (T**able 2**),

5 articles discussing TGF (**Table 3**), and 3 articles discussing endomyometrial nerve fibre (**Table 4**).

DISCUSSION

Pathogenesis of adenomyosis

There are several theories regarding the pathogenesis of adenomyosis (**Figure 2**). According to the most popular theory, adenomyosis arises from the invagination of the basalis endometrium into the myometrium, triggered by the myometrium's contractions, which cause trauma to the endometrial-myometrial junction zone (JZ), a structure that highly specialized hormone-responsive, located in the inner third of the myometrium. Persistent peristaltic myometrial contractions may induce constant microtrauma to the JZ, leading to inflammation, which in turn promotes local increases in oestrogen production and the recruitment of inflammatory mediators. Recent studies have also discovered that physiopathological mechanisms, including abnormalities in sex steroid hormones, inflammation, fibrosis, and neuroangiogenesis, may be associated to the pathogenesis of adenomyosis [10-13].

Angiogenesis is a mechanism of forming new capillaries from pre-existing blood vessels, which naturally occurs during the proliferative phase. Oestrogen plays an important role in this event through increasing cell mobilization and microvascular integration. Numerous studies have shown that increased neoangiogenesis is present in adenomyosis, as indicated by abnormalities and higher microvessel density in both ectopic and eutopic endometrium. Vascular endothelial growth factor (VEGF), a potent mitogen for endothelial cells, is highly secreted by endometrial epithelial, stromal, and perivascular cells in adenomyosis and plays a key role in angiogenesis mechanism. While VEGF is crucial for regenerating the endometrial lining after menstruation, its levels may be excessive in patients with adenomyosis [14, 15].

Additionally, two growth factors, follistatin and activin A (both part of the TGF-β family), are involved in new blood vessel formation. In adenomyosis, these factors act as proangiogenic agents by promoting the formation of new capillaries and expanding the surface area of existing ones compared to controls. Specifically, Activin A enhances VEGF production by endometrial stromal cells, altering vascularization and cause formation of

Table 1. *Summary of studies discussing VEGF as adenomyosis marker.*

Author	Year	Study Design	Sample Size	Results
Harmsen *et al.* [23]	2022	Retrospective matched case-control study	19 specimen diagnosed with adenomyosis and 19 specimen controls with unrelated pathology	There was no difference in the intensity of VEGF staining between adenomyosis and control patients
Kwack *et al.* [24]	2022	Retrospective study	A uterine sample was taken from 22 premenopausal patients with focal uterine adenomyosis. Samples were collected from three specific areas: the adenomyosis lesion, the unaffected myometrium, and the endometrial tissue just beneath the unaffected myometrium	VEGF expression was significantly higher in adenomyotic lesions and the myometrium compared to the eutopic endometrium
Yalaza *et al.* [26]	2020	Retrospective study	90 paraffin-embedded archival tissues that categorized into three groups: Group I (ectopic endometrial tissues of adenomyosis patients), (n = 35); Group II (eutopic endometrial tissues of adenomyosis patients), (n = 35); Control Group (endometrial tissues of individuals without adenomyosis), (n = 20)	There was significant difference in the level of VEGF gene expression between Group I–Group II (p = 0.036) and Group I–Control Group (p = 0.001), and there was no significant difference between Group II and Control Group (p = 0.275)
Wang *et al.* [27]	2016	Retrospective study	30 ectopic and eutopic endometrial tissues of adenomyosis patients and 10 endometrial tissues of patients without adenomyosis as control	The staining levels of VEGF in the ectopic and eutopic endometrial of patients with adenomyosis were significantly higher than in the controls
Orazov *et al.* [28]	2016	Retrospective study	Uterus specimens from 30 patients with diffuse adenomyosis accompanied by severe pelvic pain syndrome and 30 biopsies of adenomyosis patients with a painless syndrome	VEGF expression in perivascular compartment cells was found to be higher in adenomyosis patients with the painful form compared to those with the painless form
Liu *et al.* [25]	2016	Cross sectional study	Endometrial tissue specimens from 34 women with adenomyosis (excluding endometriosis) and 20 women without adenomyosis (controls)	IHC result show that staining of VEGF, were highly significantly increased in ectopic endometrium from adenomyosis patients compared to controls

VEGF: vascular endothelial growth factor; IHC: immunohistochemistry.

Table 2. *Summary of studies discussing IL-6 as adenomyosis marker.*

Author	Year	Study Design	Sample Size	Results
Jiang *et al.* [22]	2023	Retrospective study	Biopsy specimens from 10 adenomyosis patients	IL-6 expressions were detected and enhanced in adenomyosis myometrium cells that were exposed to exosomes
Kim *et al.* [30]	2019	Retrospective cohort study	Blood samples of 59 infertile women with adenomyosis	Serum IL-6 levels on the day of hCG injection were markedly higher in infertile women with adenomyosis compared to those without adenomyosis who were undergoing IVF at the same time (p = 0.01). (p = 0.01)
Jiang *et al.* [31]	2017	Retrospective study	Eutopic endometrial (EU) and Ectopic endometrial (EC) samples were derived from 30 adenomyosis patients, and endometrium samples without adenomyosis (CE) from 30 healthy patients as controls	RT-PCR analysis showed that IL-6 mRNA expression levels in EC and EU were significantly higher than in CE, with EC showing significantly higher expression than EU (p < 0.01)

IL-6: interleukin-6; hCG: human chorionic gonadotropin; IVF: in vitro fertilization; RT-PCR: reverse transcription-polymerase chain reaction; mRNA: messenger ribonucleic acid.

Table 3. *Summary of studies discussing TGF as adenomyosis marker.*

Author	Year	Study Design	Sample Size	Results
Juárez-Barber, *et al.* [34]	2022	Retrospective study	Human endometrial biopsy specimens from adenomyosis women (n = 6) and healthy women (n = 6)	Adenomyosis organoids (self-organized in vitro in 3D structures) showed there was higher expression of TGF-β2
Cheong *et al.* [37]	2019	Experimental design	Endometrium samples at secretory phase of menstrual cycle from 25 patients with adenomyosis	Expression of TGF-β1 in the stroma of adenomyotic endometrium induce collagen production in endometrium-derived fibroblasts
Cai *et al.* [36]	2019	Experimental design	Ectopic endometrial tissue samples from 40 premenopausal women with adenomyosis (28 with diffuse adenomyosis, 12 with focal adenomyosis) and endometrial samples from 40 women without endometriosis, adenomyosis, uterine fibroids	Expression of TGF-β1 was significantly elevated in adenomyosis group compared to control
Kishi *et al.* [20]	2017	Retrospective study	Biopsy specimens from 18 adenomyosis patients (8 cases occur at the inner myometrium and 10 cases occur at outer myometrium)	A significant staining of TGF-β were found only at the smooth muscle cells of subtype II adenomyosis (occur at outer myometrium)
Liu *et al.* [25]	2016	Cross sectional study	Endometrial tissue specimens from 34 women with adenomyosis (excluding endometriosis) and 20 women without adenomyosis (controls)	Adenomyotic lesions had a significantly increased staining for TGF-β1 compared to control (p < 0.001)

TGF: tumour growth factor.

Table 4. *Summary of studies discussing endomyometrial nerve fibre as adenomyosis marker.*

Author	Year	Study Design	Sample Size	Results
Yadav *et al.* [39]	2021	Prospective study	Endometrial tissue specimens of 190 patients with endometriosis, adenomyosis, or uterine fibroids (73 patients had adenomyosis) and 30 patients without endometriosis, adenomyosis, uterine fibroids	There were 10/73 (13.7%) patients in the adenomyosis group who had endomyometrial nerve fibres and a significant difference was observed in the presence of nerve fibres among these groups (endometriosis, adenomyosis, or uterine fibroid, p <0.001)
Takeuchi *et al.* [40]	2016	Experimental Design	Adenomyosis tissue samples from 12 patients divided into 6 patients who received dienogest and 6 patients who did not receive hormonal treatment for ≥3 months as the control group	The density of nerve fibres in adenomyosis lesions was significantly reduced in the dienogest group compared to the control group
Lertvikool *et al.* [42]	2014	Cross sectional study	Uterine samples from 23 reproductive age women with adenomyosis that divided into two groups, VAS ≥ 5 (moderated and severe pain) and VAS < 5 (less pain)	Nerve fibres density was significantly higher in adenomyosis patients with moderate and severe pain compared to less pain group

VAS: visual analogue scale.

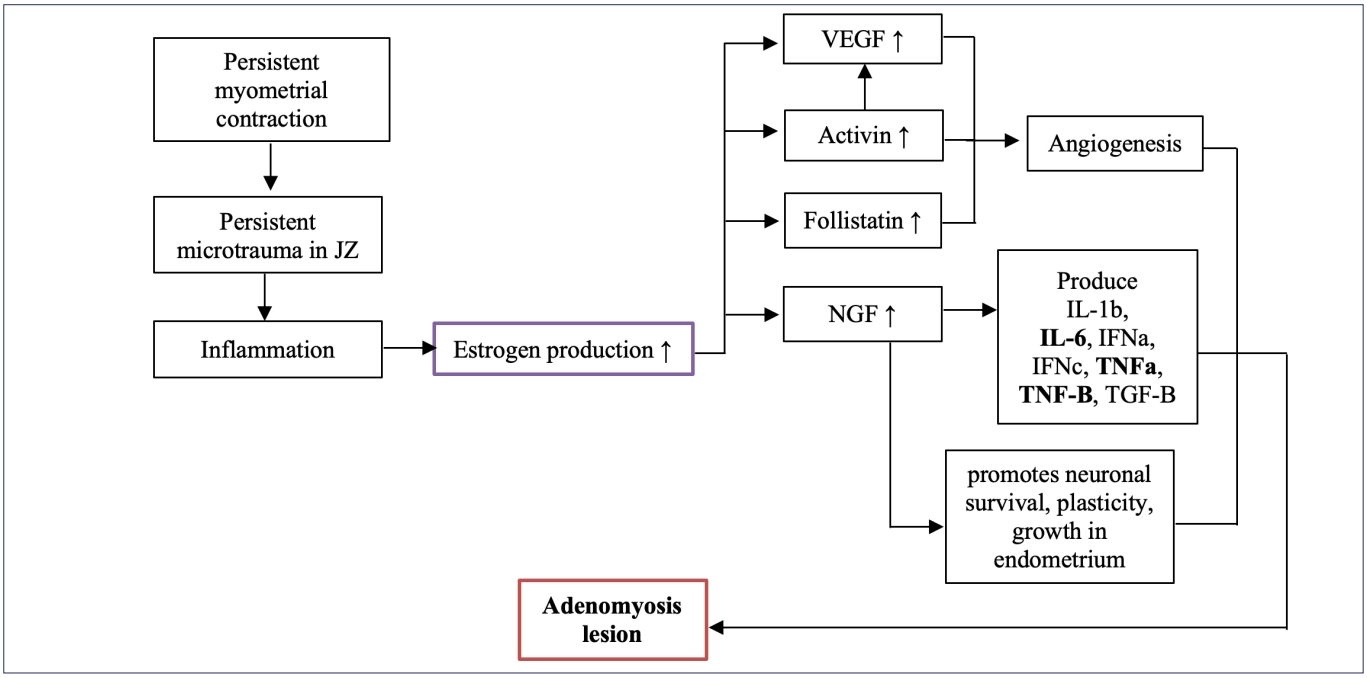

Figure 2. *Overview of pathogenesis of adenomyosis.*

new capillary. Furthermore, the mRNA expression levels of follistatin and activin type II receptors are elevated in adenomyotic nodules [11].

The increasing expression of oestrogen and TNF also trigger adenomyotic tissues to produce elevated levels of neurogenic factors, like nerve growth factors (NGF), which control the secretion of inflammatory factors, leading to mast cell growth and degranulation, producing inflammatory mediators, including IL-1b, IFNa, IFNc, IL-6, TNFa, TNF-B, TGF-B.28,30 Additionally, NGF promotes neuronal survival, plasticity, growth, and differentiation of catecholamine production in a manner that depends on the dose. Zhang *et al*. reported finding nerve fibres in the functional layer of the endometrium in women with adenomyosis who experienced pain symptoms. These nerve fibres were absent in women with asymptomatic adenomyosis. NGF may be related to these findings [11].

Menstrual blood-based biomarker

Several biomarkers have been investigated in research for diagnosing adenomyosis, but none have been implemented in clinical practice. Menstruation is the process of shedding the functional layer of the uterine lining following the luteal phase of the ovarian cycle. Endometrium is a multicellular and dynamic uterine tissue that is highly responsive to sex steroid hormones [16]. Menstrual blood offers a promising non-invasive diagnostic tool because layers of the endometrium are shed during menstruation and returned to the pelvic cavity during menstruation, simplifying and accelerating the diagnostic process [17]. Some of these biomarkers can be found in menstrual blood, including VEGF. VEGF protein is expressed in normal endometrial stromal cells, with levels rising in response to oestrogen and progesterone, which are elevated in adenomyosis [18]. Sex hormones that regulate the menstrual cycle also induce the secretion of various cytokines (such as interleukin and TGF) in the uterine endometrium, which are essential for angiogenesis, the proliferation of natural killer (NK) and T cells, decidualization, and implantation [19]. Thus, these cytokines can thus be found in menstrual blood. Additionally, nerve fibres can be detected in menstrual blood, both under physiological and pathological conditions. In pathological conditions like adenomyosis, where endometrial tissue infiltrates the muscular layer of the uterus, nerve fibres can also be released during menstruation [20]. While still under research, evidence for menstrual blood biomarkers may one day be as robust as that for biomarkers used in cervical cancer [21].

Study by Burghaus *et al*. also revealed there are other potential biomarkers for adenomyosis. Burghaus *et al*. found compared to 5 other blood-based biomarkers (HGF.aAB, Prokineticin-1, NSE, S100-A12, DNASE2.aAB), sFRP-4 was the best performing univariate biomarker with a sensitivity of 56.4% for

comparison *versus* "all symptom controls". For comparison *versus* "pathology-free symptom control", S100-A12 was the best performing univariate biomarker with a sensitivity of 74.6% [8].

VEGF as biomarker of adenomyosis

In adenomyosis, tissue injury and repair result in the accumulation of myofibroblasts in the affected myometrium, causing myometrial hypertrophy. VEGF plays a significant role in stimulating the growth of new blood vessels, supplying oxygen and nutrients to the proliferating tissue. In adenomyosis, additionally, repetitive tissue injury causes local vascular disruption and blood extravasation, leading to platelet aggregation, the formation of clots, and consequent hypoxia. In response to hypoxic stimuli, hypoxia-inducible factor-1alpha (HIF-1α), a main mediator of cellular adaptation to hypoxia, is activated [18, 22].

Macrophages also recruited to the wounding site, secrete chemotactic factors and several growth factors, including VEGF. This factor is important for cell migration and proliferation, which mediates tissue repair and is enhanced by activation of platelets releasing a series of cell growth factors and angiogenic factors, such as PDGF and VEGF. VEGF plays a crucial role in regenerating the endometrial layer after menstruation; however, it is overexpressed in patients with adenomyosis. Consequently, an increase in VEGF expression is anticipated in the myometrium of adenomyosis patients [18, 22]. While studies utilizing immunochemistry (IHC) and RT-PCR have demonstrated increased VEGF expression in adenomyotic lesions, inconsistencies remain. For instance, Harmsen *et al*. reported no difference in myometrial VEGF levels between patients with adenomyosis and control. The study also found that IHC score of VEGF was highest in the myometrium, followed by endometrial glands, and lowest in the endometrial stroma. While their study was the first to utilize multiplex IHC, the limited number of sample is a clear limitation. The samples were also only analysed based on areas of interest, which may not be representative [23]. In contrast to the result by Harmsen [23], Kwack *et al*. [24], Liu *et al*. [25], and Yalaza *et al*. [26] all also found VEGF expression levels to be elevated in adenomyotic and myometrial lesions compared with eutopic endometrium, whether from normal subjects or those with adenomyosis.

Wang *et al*. also reported similar findings, with notably higher levels of VEGF in the endometrial glandular epithelial cells of adenomyotic lesions. Additionally, the study found that the immunoreactivity of GRIM-19, a novel protein which regulates apoptosis and the formation of new blood vessels, was markedly reduced in the adenomyosis group. In adenomyosis, deficiency in expression of GRIM-19 results in decreased apoptosis and increased angiogenesis [27]. Orazov *et al*. also added that VEGF levels were significantly higher in ectopic endometrial epithelial cell and in the myometrial smooth muscle cells and stromal cells. VEGF levels were also reported to be higher than those found in abnormal uterine bleeding. These findings suggest that the neovascularization process, which is promoted by VEGF, plays a significant role in the development of pelvic pain associated with adenomyosis [28]. Future studies should further study the role of VEGF in the pathophysiology of adenomyosis, along with the role of novel proteins, such as GRIM-19. Utilization of advanced techniques such as multiplex IHC is also promising. However, retrospective design of the studies may introduce selection bias and should be addressed in subsequent studies.

IL-6 as biomarker of adenomyosis

IL-6 is a growth regulator of human endometrial stromal cells. When bound to its receptor, it activates JAK2, leading to the phosphorylation and nuclear localization of signal transducer and activation of transcription 3 (STAT3). This signalling pathway is crucial for the growth and progression of various human cancers, including endometrial carcinoma. Hyperactivation of this signalling pathway may enhance the invasive behaviour of endometrial cells in adenomyosis. Exosomes, a subtype of extracellular vesicles, function as carriers for transferring molecules such as DNA, RNA, proteins, and lipids from parental cells to recipient cells. Thus, endometrial cell-derived exosomes could facilitate communication between the endometrium and myometrium via IL-6 signalling, playing a role in the development of adenomyosis [22, 29].

Jiang *et al*. found that IL-6 expressions were enhanced in adenomyosis myometrium cells that were exposed to exosomes. Western blotting also revealed that endometrial cell exosomes are significantly increased the protein expression of IL-6, p-JAK2, JAK2, p-STAT3, STAT3, which influenced the effect of endometrial cell exosomes on AM cells. Reflecting on this, exosome inhibitors may be a future therapeutic modality in adenomyosis. IL-6

may also be directly targeted in future treatment of adenomyosis. Adenomyotic myometrium cells exposed to tocilizumab, an IL-6 inhibitor, were also observed to display apoptotic characteristics and significant reduction in survivability during the MTT assay [22]. However, there are still questions to be answered with regards to the precise mechanisms of tocilizumab and its dynamics on immune cells in adenomyosis.

Study by Kim *et al*. in patients undergoing IVF revealed higher baseline IL-6 levels in infertile adenomyosis patients with clinical pregnancy rate being significantly lower in those with higher IL-6 levels [30]. It would be interesting to explore the prognostic role of IL-6 in pregnancy and fertility among patients with adenomyosis.

Jiang *et al*. also reported RT-PCR results that showed IL-6 mRNA expression levels in ectopic and eutopic were significantly higher than in control group. Jiang *et al*. also found a significant positive correlation between IL-6 mRNA expression and TLR-1,4,5, and 9 in eutopic tissue. In EC, IL-6 mRNA expression was positively correlated with TLR-1, 2, 4, 5, 6, and 9, but did not show any significant correlation with other TLRs. These findings suggest that TLRs might potentially play a role in the inflammatory development of adenomyosis through the NK-κB-mediated signalling pathway [31, 32].

TGF as biomarker of adenomyosis

The Epithelial-mesenchymal transition (EMT) is a physiological process where epithelial cells gain the motile and invasive properties of mesenchymal cells. During embryonic development, EMT is an expected and coordinated process which involves interactions among various cells and tissues [33]. However, microenvironmental changes and abnormal stimuli may improperly activate the EMT process, contributing to the pathogenesis of adenomyosis. Transforming growth factor (TGF)-β1 and TGF-β2 may play an important role in the induction and regulation of EMT. The upregulation of these factors in the endometrium of patients with adenomyosis indicate a dysfunction during the secretory phase [34, 35].

Research conducted by Juárez-Barber *et al*. supported this notion as they reported significantly increased TGF-β2 expression in adenomyosis when evaluated by IHC [34], with Cai *et al*. and Liu *et al*. reporting comparable results [25, 36]. Furthermore, the experiment by Cai *et al*. found a negative correlation between the level of eIF3e staining and TGF-β1. According to recent research, decreased expression of eIF3e is associated with the epithelial-mesenchymal transition (EMT) process. The phenomena of EMT may be a widespread occurrence in disease progression and requires an active and ongoing TGF-β signalling pathway, which may also be a future therapeutic target with antibodies or small molecule inhibitors [36].

Results from the experiment by Cheong *et al*. suggested that TGFβ1 influences collagen production by inducing CTGF, a protein classified within the CCN family of matricellular proteins. It is a key regulator of tissue remodelling and fibrosis where impairment causes excessive extracellular matrix (ECM) synthesis which is implicated in various fibrotic conditions [37]. Elevated levels of CTGF may promote the development and fibrotic advancement of adenomyosis, which consequently lead to dysmenorrhea [38].

Endomyometrial nerve fibre as biomarker of adenomyosis

Recent research has identified fine and unmyelinated sensory nerve fibres in the functional layer of the eutopic endometrium in women with endometriosis. These nerve fibres have subsequently been observed in the peritoneal endometrioses. Study by Yadav *et al*. reported that 10 out of 73 patients (13.7%) in the adenomyosis group had nerve fibres, as indicated by positive PGP 9.5 staining. However, the percentage of women with endomyometrial nerve fibres is significantly higher in the endometriosis group [39].

Research by Takeuchi *et al.* in adenomyosis patients found significantly lower density of nerve fibres and lower NGF immunoreactivity in those receiving dienogest [40]. Dienogest, a novel progestin derived from 19-norsteroid, is highly selective for progesterone receptors and exhibits antiproliferative, immunologic, and antiangiogenic effects on endometrial tissue. It also significantly reduces chronic pelvic pain and menorrhagia in patients with adenomyosis [41]. Furthermore, Lertvikool *et al*. reported significantly increased number of nerve fibres identified by PGP9.5 staining in the myometrium of adenomyosis patients experiencing moderate to severe pain when compared to those with less pain. NGF and its receptors are crucial in mediating both neuropathological and non-neuropathological pain by promoting the growth, survival, and maintenance of sensory neurons. Studies in a mouse model have also shown that NGF-beta

is a key factor in the pathogenic mechanisms of adenomyosis [42]. These findings shed a light on the possible mechanism on how dienogest may reduce pain in adenomyosis.

Clinical implications

There are three primary ways in which measuring a biomarker in clinical care can enhance health: it can help the patients in understanding their disease, which enhances their quality of life and mental health; it can motivate patients to adopt healthier behaviours, such as better diet, increased exercise, or improved adherence to prescribed treatments; and it can assist clinicians in making better clinical decisions, such as determining appropriate treatments, which leads to better patient health. However, biomarker measurements can also have negative health outcomes through these same mechanisms (for example causing depressed mood from unfavourable news). Furthermore, these biomarkers may also have the potential to provide gynaecologic and obstetric prognostic value in the holistic management of adenomyosis. Therefore, before ordering a biomarker test, clinicians should have a clear expectation that, on average, the test will lead to improved health through one or more of these mechanisms.

CONCLUSIONS

Most of the research results point to the possibility of VEGF, IL-6, TGF, and endomyometrial nerve fibres as potential biomarkers for adenomyosis. In summary, we found these expressions were significantly increased in patients with adenomyosis compared to the control group (without adenomyosis). VEGF and endomyometrial nerve fibres may play an important role in pain in adenomyosis [43,44]. However, more evidence backed by better research methodology is still necessary before routine clinical application, such as by employing an experimental design and blinding. This would also better resolve the conflicting findings seen between authors.

COMPLIANCE WITH ETHICAL STANDARDS

Authors' contribution

A.R.: Conceptualization. Y.I.A.: Data curation, formal analysis, investigation, project administration, resources, software, visualization, writing - original draft, writing - review & editing. D.T.: Methodology. A.R., D.T.: Supervision, validation.

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Fractional CO_2 laser treatment for vaginal rejuvenation: a narrative review and comprehensive update

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ABSTRACT

Vulvovaginal atrophy (VVA) and genitourinary syndrome of menopause (GSM) are common conditions affecting postmenopausal women, leading to symptoms such as dryness, itching, and dyspareunia. Traditional treatments include hormonal therapies and lubricants, but non-hormonal options have newly emerged. Vaginal rejuvenation has gained popularity in recent years, addressing a range of conditions related to aging, childbirth, and hormonal changes. Fractional carbon dioxide (CO_2) laser treatment offers a minimally invasive approach to improve vaginal health, promoting collagen synthesis and enhancing tissue elasticity. Fractional CO_2 lasers work by delivering controlled thermal energy to the vaginal tissue, creating micro-injuries that stimulate the body's natural healing processes. These changes correlate with clinical improvements in vaginal hydration and pH levels. We emphasize the significant role of fractional CO_2 laser treatment in vaginal rejuvenation in postmenopausal women with GSM. It offers a safe, minimally invasive, and effective option to improve their quality of life and sexual health. This review explores the mechanisms, clinical applications, safety, and patient satisfaction associated with fractional CO_2 laser therapy, aiming to comprehensively understand its role in contemporary gynaecological practice.

INTRODUCTION

Vaginal health is integral to overall quality of life, with many women experiencing vulvovaginal atrophy (VVA) issues such as dryness, burning, or itching, particularly after menopause, owing to estrogen insufficiency. These symptoms are often progressive and increase over time as hypoestrogenism persists. However, it is sometimes referred to as a "silent symptom of menopause". VVA can also be caused by iatrogenic events such as surgery and radio or chemotherapy in cancer cases. The medical term "vulvovaginal atrophy" has been replaced by the most recent "genitourinary syndrome of menopause" (GSM) to cover other vaginal, sexual, and urinary symptoms caused by the estrogen insufficiency such as dysuria, urinary frequency, urethral discomfort, haematuria, sexual discomfort

or dyspareunia. This disorder affects up to 60% of postmenopausal women, and it has a significant impact on quality of life and sexual function, being frequently overlooked and undertreated [1-3].

Traditional non-surgical treatments include systemic hormones and topical hormonal and non-hormonal treatments. For moderate to severe symptoms, estrogen treatment is the most effective therapeutic option; non-hormonal remedies relieve minor symptoms as well as other menopausal symptoms [4]. Systemic estrogen therapy is sometimes used; however, many women prefer not to use hormonal treatment due to adverse effects, for example, increased symptoms of urine incontinence. Moreover, 10% - 20% of women may have remaining GSM symptoms. Local estrogen treatment remains controversial as it appears to recover symptoms more efficiently. However, there is insufficient evidence to prove the safety of local estrogen, and endometrial safety has not been explored in long-term clinical investigations, especially in breast cancer patients. On the other hand, non-hormonal local moisturizers and lubricants can be safe and efficient in relieving GSM symptoms; however, they must be used on a regular basis for maximum results [5-9].

Traditional surgical options, while effective, can be invasive and carry significant recovery times. Vaginal rejuvenation encompasses a variety of procedures aimed at enhancing the function, appearance, and overall health of the vaginal area. Light amplification by stimulated emission of radiation (LASER) technology emerges as a promising alternative treatment for postmenopausal vaginal symptoms, offering minimal downtime and a less invasive approach, especially in women who are pursuing non-hormonal alternatives. Studies reveal that laser technology may also aid people who suffer from vulvar *Lichen sclerosus* (LS) [10, 11].

Laser methods to treat vulvovaginal problems employ a wavelength that may significantly absorb water, such as the 10,600 nm of the carbon dioxide (CO_2) laser, in order to coagulate and ablate the vulvar and vaginal tissues. Through thermal diffusion, the Er-YAG laser also produces non-ablative photothermal effects in the vaginal walls. Heating sets off a wound response that causes atrophied skin to restructure and produce new collagen and elastic fibres [12, 13]. The vulvovaginal complex's supporting tissues are then tightened to restore vaginal muscle tone. CO_2 laser therapy has already been shown to significantly alleviate GSM symptoms in postmenopausal women. Fractional CO_2 laser treatment has also been proven to improve vaginal mucosa structure in postmenopausal or non-estrogenised women [14-18].

Although fractional CO_2 laser treatment may exert minimal adverse effects, it provides a safe therapeutic approach for VVA. Patients are usually pleased and express a desire to have laser therapy again because serious issues rarely occur, especially with trained professionals performing procedures, and the incidence of moderate problems is small and usually resolved without the need for therapy. Therefore, the safety profile of fractional CO_2 laser is generally favourable [19].

This study explores the use of fractional CO_2 laser for vaginal rejuvenation in cases of GSM, focusing on its mechanism, associated histological changes, clinical applications, efficacy, safety, and patient satisfaction.

MECHANISM OF ACTION

Fractional CO_2 lasers utilize a specific wavelength of light to target the vaginal mucosa, inducing thermal injury in a controlled manner. This stimulates fibroblast activity and collagen production, leading to tissue tightening and rejuvenation. Unlike conventional lasers, fractional delivery allows for the preservation of surrounding tissues, reducing recovery time and discomfort [20].

The application of fractional CO_2 lasers in gynaecological procedures has been shown to improve symptoms related to vaginal atrophy, urinary incontinence, and overall vaginal health [21]. Clinical studies have demonstrated significant improvements in patient-reported outcomes, indicating the efficacy and safety of this minimally invasive treatment option [22].

Fractional CO_2 lasers deliver controlled thermal energy to the vaginal tissue, creating micro-injuries that trigger the natural wound-healing response of the body. This technology has been widely researched and is recognized for its effectiveness in promoting vaginal rejuvenation and overall tissue health. The key mechanisms include:

1. Thermal energy delivery:
    - Micro-injuries: the laser creates microscopic columns of thermal damage in the vaginal mucosa, sparing surrounding tissue and promoting rapid healing. This process is essential as it stimulates the natural healing pro-

cesses of the body, leading to the production of new collagen and elastin fibres, which are crucial for maintaining the structural integrity and elasticity of vaginal tissue [15].
- Controlled ablation: this process removes damaged tissue and stimulates healthy tissue regeneration. The controlled ablation facilitated by the CO_2 laser ensures the precise removal of aged or damaged cells while minimizing the risk of adverse effects. Therefore, it stimulates cellular turnover and enhances tissue quality and function [23].

2. Collagen production:
   - Stimulation of fibroblasts: the thermal effect of laser activates fibroblasts to produce collagen and other extracellular matrix components, playing a vital role in tissue repair and regeneration [24].
   - New collagen fibres: increased production of collagen fibres enhances tissue elasticity and firmness. Collagen is a key structural protein in the skin and other connective tissues, providing strength and support [24].
3. Tissue remodelling:
   - Wound healing response: the micro-injuries trigger a wound healing response, leading to healthy tissue regeneration. This process involves the activation of various cellular and molecular pathways that promote tissue repair and regeneration [25].
   - Angiogenesis: enhanced blood flow and new blood vessel formation improve tissue oxygenation and nutrient delivery. Angiogenesis is a critical component of the healing process, as it ensures that the newly formed tissue receives adequate oxygen and nutrients for optimal growth and repair [26, 27].
4. Improved lubrication and vaginal secretions: enhanced blood flow and collagen production lead to better lubrication, reducing dryness and discomfort. The increased blood flow and collagen production stimulate the production of vaginal secretions, which helps in maintaining proper lubrication and alleviating symptoms of dryness and discomfort [20].

HISTOLOGICAL CHANGES

Histological studies have provided insights into the tissue-level changes induced by fractional CO_2 laser treatment:

1. Collagen and elastin:
   - Increased staining: studies have shown increased staining for collagen and elastin fibres, enhancing tissue strength and elasticity. This increased staining reflects the higher presence of these structural proteins, which are essential for maintaining skin resilience and flexibility [28].
   - New collagen fibres: the treatment stimulates the production of new collagen fibres, contributing to improved tissue structure. The formation of new collagen fibres is a key factor in the rejuvenation process, as it helps restore the integrity and firmness of the treated tissue [29].
2. Epithelial thickness:
   - Thicker epithelium: the treatment leads to a thicker epithelium, which correlates with improved vaginal hydration and pH levels. This increase in epithelial thickness is associated with better tissue health and function [30].
   - Increased cell layers: histological findings show increased cell layers and a better degree of surface maturation. This indicates a more robust and well-structured epithelial layer, contributing to overall tissue integrity [31].
3. Angiogenesis:
   - New blood vessels: enhanced blood flow and new blood vessel formation improve tissue oxygenation and nutrient delivery. This process is essential for maintaining healthy tissue and promoting healing [26, 27].
   - Improved vascularization: the treatment promotes angiogenesis, which is crucial for tissue health and repair. Increased vascularization ensures that the treated area receives adequate oxygen and nutrients, supporting healing [26, 27].
4. Improved surface maturation: the treatment enhances the surface maturation of the vaginal epithelium, contributing to better overall vaginal health. This improved maturation results in a more robust and well-structured epithelial layer, essential for maintaining tissue integrity and function [20].

CLINICAL APPLICATIONS AND EFFICACY

Studies have shown that fractional CO_2 laser treatment can effectively improve symptoms of vaginal atrophy, enhance sexual function, and even reduce

urinary incontinence. These studies have demonstrated significant improvements in vaginal health scores, with many patients reporting enhanced satisfaction in their intimate relationships.

Gaspar *et al*. (2011) studied the effect of combined CO_2 laser, platelet-rich plasma (PRP), and pelvic exercise (study group) *vs* combined PRP and pelvic exercise (control group) on vaginal dryness, dyspareunia, and itching. They found significant improvement in vaginal mucous histology and sexual function in most cases in the study group [22].

A study was conducted by Arroyo in 2017 on 21 perimenopausal women with VVA symptoms. They received three sessions of fractional CO_2 laser for resurfacing and coagulating the mucosal lining of the vagina and the introitus. Vaginal health index (VHI) scores and visual analogue scale (VAS) showed an improvement in vaginal health and relief of the vulvovaginal symptoms as well as improved sexual function in perimenopausal women [20].

Ruanphoo and Bunyavejchevin (2020) conducted a randomized double-blinded sham-controlled trial on 88 postmenopausal women with moderate to severe symptoms of vaginal atrophy. They received either microablative fractional CO_2 laser or sham procedures three times four weeks apart. After 12 weeks of treatment, the VHI and VAS scores were significantly improved in the laser group compared to the control group [32].

Salvatore *et al*. (2021) studied the effect of microablative fractional CO_2 laser for treating vulvovaginal symptoms in 40 women with a history of breast cancer. There was marked improvement in vulvovaginal symptoms and sexual function, with a significant decrease in VAS score and a significant increase in vaginal health index score (VHIS) and female sexual function index (FSFI). Their prospective cohort study concluded that microablative fractional CO_2 laser is a safe and effective treatment option for women on endocrine therapy, either previously or currently [33].

Gardner and Aschkenazi (2021) conducted a retrospective study on 139 menopausal women with breast cancer and *Lichen sclerosus* and vulvovaginal symptoms after CO_2 laser therapy every six weeks for three sessions. They concluded that treatment for VVA with fractional CO_2 laser is safe and successful as they found that all FSFI and VAS scores showed significant improvement with no major harmful events reported [34].

A systematic review was conducted by D'Oria *et al*. (2022) to evaluate the therapeutic efficiency of fractional CO_2 laser for VVA in young women with a history of gynaecological cancer. They concluded that fractional CO_2 laser is an effective and safe therapeutic choice for young women with a history of gynaecological cancer, as it improves the quality of life and sexual satisfaction [35].

Donato *et al*. (2022) conducted a cohort study on 92 menopausal women with VVA to assess the efficacy of fractional CO_2 laser therapy. They concluded that fractional CO_2 laser improves vaginal health as well as GSM-related signs and symptoms while also dramatically increasing the quality of life and sexual functioning in postmenopausal symptomatic women [36].

Jankovic *et al*. (2024) conducted a cohort study on 84 sexually active postmenopausal women to assess the effects of fractional CO_2 laser on the clinical symptoms of VVA (evaluated by VAS and VHIS) and the sexual function (evaluated by FSFI). They found a significant decrease in VAS score and a significant increase in VHIS and FSFI, denoting marked improvement in symptoms of VVA and sexual function [37].

Adabi *et al*. (2024) conducted a prospective study on 140 postmenopausal women with vaginal atrophy to assess the impact of the fractional CO_2 laser on their quality of life, vaginal atrophy symptoms, and urinary incontinence. They found significant improvement in the quality of life regarding somatic, social function, and mental health. In addition, sexual arousal, satisfaction, and urinary symptoms showed marked improvement [38].

On the contrary, Li *et al*. (2021) conducted a double-blinded randomized clinical trial to examine the efficacy of CO_2 laser therapy on vaginal symptoms related to menopause in 90 women with postmenopausal symptoms. They found no significant difference after 12 months between the CO_2 laser group and the control group in the change in VAS score for total vaginal symptoms, mean quality of life score, and VHIS [39].

SAFETY AND COMPLICATIONS

While generally considered safe, fractional CO_2 laser treatment can have side effects, including transient discomfort, edema, and, rarely, infections. The safety profile of fractional CO_2 laser treatment is generally favourable, with most studies reporting

minimal adverse effects. A thorough review of existing literature highlights a low incidence of severe complications, particularly when trained professionals perform procedures.
For example, a study by Gaspar *et al*. (2011) reported that 30% of patients experienced transient discomfort and edema following fractional CO_2 laser treatment for vaginal rejuvenation that resolved within 5 days of applying diclofenac gel locally once daily [22]. Another study by Arroyo (2017) noted that only one case had a mild urinary infection after fractional CO_2 laser, and she completely recovered by using antibiotics orally for one week [20]. Additionally, in 2020, Di Donato concluded in his study that fractional CO_2 laser provides a safe therapeutic approach for VVA. They found that 94.9% of patients were pleased and expressed a desire to have laser therapy again. This could be attributed to rare serious issues, especially with trained professionals performing procedures, and the incidence of moderate problems was small and resolved without needing therapy [19].
On the other hand, women who received traditional surgical interventions may carry a higher risk of infection that may require additional treatment. In addition, surgical procedures often require a longer recovery period, during which patients may experience pain and limited mobility. However, Surgical interventions, such as vaginoplasty and perineoplasty, could be more efficient for severe to moderate degrees of vaginal laxity. Therefore, the severity of vaginal laxity should be considered before selecting the appropriate approach for vaginal rejuvenation [40].

PATIENT SATISFACTION

Patient satisfaction with fractional CO_2 laser treatment is generally high, with many women reporting significant improvements in symptoms and quality of life. Research indicates high patient satisfaction following treatment, and many women report improved self-esteem and quality of life. Patients often experience enhanced sexual function, reduced discomfort, and improved overall well-being. Furthermore, it was reported that patient satisfaction remains high even 12 months post-treatment, highlighting the long-term benefits of the fractional CO_2 laser treatment. Factors such as pre-treatment counselling and realistic expectation setting play crucial roles in overall satisfaction.
A study by Woźniak *et al*. (2023) evaluated the clinical effectiveness of fractional CO_2 laser in treating GSM symptoms and used a treatment satisfaction questionnaire. The results showed significant symptom improvement and high patient satisfaction [41]. Another randomized clinical trial was conducted by Mension *et al.* (2023) on 84 patients with GSM and a history of breast cancer. This study reported high levels of patient satisfaction with the fractional CO_2 laser treatment [42]. A systematic review has also discussed the efficacy and patient satisfaction with fractional CO_2 laser treatment for vulvovaginal rejuvenation, often used to treat symptoms of GSM [43].

CONCLUSIONS AND RECOMMENDATIONS

Fractional CO_2 laser treatment represents a significant advancement in vaginal rejuvenation, offering a safe, minimally invasive, and effective option for many women experiencing symptoms associated with GSM. Fractional CO_2 laser therapy should be the mainstay in gynaecological practice, at least in postmenopausal women with mild GSM and breast or gynaecological cancer survivors who suffer from VVA and are not eligible for hormonal treatment.
While current literature demonstrates the efficacy of fractional CO_2 laser therapy for vaginal rejuvenation, there is a need for further research to establish definitive treatment protocols for different severity of VVA and menopausal stages and identify the most suitable device. Additionally, clinical trials with long-term follow-up are needed to compare fractional CO_2 laser therapy with the other surgical options, such as labiaplasty or vaginoplasty, regarding the outcome and cost-effectiveness to ensure that women receive the most appropriate and beneficial treatment for their individual needs.

COMPLIANCE WITH ETHICAL STANDARDS

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Evaluating the effectiveness of castor oil for labour induction: a narrative review

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ABSTRACT

Background and Objectives. The process of artificially stimulating the uterus to initiate labour is commonly referred to as labour induction. This procedure should be offered to women only when supported by scientific evidence demonstrating that the benefits of initiating labour early outweigh the associated risks. These risks include complications related to prematurity or post-term pregnancy. Various methods for inducing labour are available, categorized into pharmacological and mechanical approaches. Among pharmacological methods, the administration of exogenous prostaglandins such as Dinoprostone and misoprostol is the most widely used. Mechanical methods include transcervical catheters, amniotomy, and membrane sweeping. Additionally, international guidelines mention several "non-traditional" methods, such as acupuncture, herbal remedies, homeopathy, hot baths, enemas, sexual activity, and castor oil. The objective of this review is to evaluate the effectiveness of castor oil as a method for inducing labour.

Methods. A bibliographic search was conducted using three biomedical databases: PubMed, Embase, and CINAHL. The research question was formulated using the PIO (Population, Intervention, Outcome) framework.

Results. The most recent clinical guidelines advise against the routine use of castor oil for labour induction. However, some studies have reported its effectiveness as a non-traditional method for initiating labour.

Conclusions. Given the demonstrated effectiveness of castor oil in stimulating uterine contractions, its low cost, and the lack of significant side effects associated with its use, this method remains an area of interest for further research.

INTRODUCTION

The procedure of artificially stimulating the uterus to induce labour is commonly referred as induction of labour (IOL) [1]. This technique aims to stimulate uterine contractions before the start of spontaneous labour and is indicated when the maternal and perinatal risks of continuing pregnancy outweigh those associated with expedited birth [2]. IOL is frequently used in birth centres worldwide. Its use must be clinically justified and carefully evaluated since the risks of IOL itself [3]. The indications for IOL can be categorized into high-priority indications (chorioamnionitis, preeclampsia, post-term pregnancy, significant maternal illness, antepartum haemorrhage, foetal compromise, rupture of membranes) and other indications [4].

The frequency of IOL has increased in recent decades. In developed countries, 20 to 25% of women undergo IOL annually [5]. In Italy, the latest national data available indicates a percentage of induced births at 31.5% [6].

Before the starts of any labour's changes, the uterine cervix is approximately three and a half centimetres long. It is mainly composed of collagen and 10-15% of smooth muscle. Numerous changes must occur to initiate labour and allow dilatation of the cervical canal [7]. A wide range of methods is available for labour induction, each with different mechanisms of action, side effects, costs, duration, need for continuous maternal-foetal monitoring, and varying resource usage. The choice of one method over another depends on the indication for induction, guidelines and protocols, urgency to achieve delivery, clinical factors, and the preferences of both the woman and the healthcare provider [8].

Induction methods are divided into pharmacological and mechanical: among the pharmacological methods, prostaglandins are commonly used as they induce rapid cervical dilatation; however, they require hospital admission and continuous monitoring of side effects, especially foetal tachycardia [5]. The use of prostaglandins is recommended for inducing labour in women with an unfavourable cervix (Bishop score < 6). Several Cochrane reviews [7, 8] have demonstrated the effectiveness of PGE2 in its various formulations compared to placebo, particularly in achieving delivery within 24 hours [9, 10]. Synthetic oxytocin is also widely used, despite possible negative effects such as increased infection risk for the mother and baby, abnormal uterine contractions, higher incidence of instrumental delivery, uterine hyperstimulation, and lower maternal satisfaction with the birth experience [11]. Among the traditional methods, the use of oral misoprostol, a synthetic analogue of prostaglandin E1, has also proven effective in inducing labour [12, 13]. Initially used for the prevention and treatment of gastric ulcers and generally to prevent damage to the gastrointestinal mucosa, misoprostol is an inexpensive drug, stable at room temperature, and available in many countries worldwide, making it particularly useful in resource-poor settings [14]. For this reason, the World Health Organization has included misoprostol in the list of essential medicines [15]. Misoprostol acts on the cervix, facilitating cervical dilation and simultaneously promoting uterine contractions [16]. There is an extensive bibliography demonstrating the efficacy and superiority of misoprostol compared to other PGE2-based drugs. Specifically, misoprostol shows greater effectiveness in reducing the time between induction and delivery, increasing the likelihood of achieving vaginal delivery within 24 hours, ensuring greater safety in the case of premature rupture of membranes, and reducing the risk of caesarean section [17]. On the other hand, Dinoprostone is a synthetic preparation chemically and structurally identical to prostaglandin E2 (PGE2), which is naturally present in maternal tissues, particularly in the placenta, uterus, amniochorion membranes, and cervix. Its primary local effects include changes in cervical consistency, dilation, and effacement, as well as indirectly inducing uterine contractile activity by stimulating the myometrial response to endogenous or exogenous oxytocin [18].

Regarding mechanical methods, including the use of transcervical catheters, amniotomy, and membrane sweeping, numerous studies [19, 20] in recent years have demonstrated their efficacy and safety for pre-induction of labour in the case of an unfavourable obstetric finding. Mechanical methods are believed to work by stimulating the endogenous production of prostaglandins through the stretching of amniochorion membranes and myometrial cells, and by promoting the production of endogenous oxytocin via the Ferguson reflex. Current literature data [21, 22] have highlighted a comparable rate of caesarean sections to the use of prostaglandins (PGE2), similar efficacy to prostaglandins, a reduced risk of uterine hyperstimulation with foetal heart rate alterations compa-

red to prostaglandins, a reduced risk of caesarean sections compared to the use of oxytocin, and a good safety profile in women with previous caesarean sections.
Additionally, it should be noted that mechanical methods are all low-cost. Among mechanical methods, the most widely used is the balloon catheter. The advantages of using the balloon catheter are the possibility of employing it in an outpatient setting, which results in a reduction in hospital stay and a decrease in the caesarean delivery rate [5]. Additionally, it does not require continuous monitoring and reduces the risk of uterine hyperstimulation [5].
International guidelines also mention a series of "non-traditional" methods such as acupuncture, herbs, homeopathy, hot baths, enemas, sexual activity, and castor oil. Recent indications report that the available evidence does not support the use of these methods for labour induction [8].
Given these premises, the aim of this study is to provide a response regarding the effectiveness of using castor oil as a method for inducing labour.

MATERIALS AND METHODS

For the bibliographic search, three biomedical databases – PubMed, Embase, and CINAHL – were consulted. No time restrictions were applied to ensure the search strategy was as inclusive as possible and aligned with the study's objectives. The research question was developed using the PIO framework, defined as follows:

- P (Population): pregnant women requiring labour induction for obstetric reasons.
- I (Intervention): use of castor oil.
- O (Outcome): induction and initiation of labour.

Only articles in English, including case report were included in the review. No restrictions were applied regarding the population's age, gestational age at induction, the presence of obstetric pathologies, or any specific obstetric conditions. Regarding the intervention, all methods of castor oil administration were considered, including variations in dosage and timing. The application of the PIO framework facilitated evidence synthesis and contributed to the development of this narrative review. SANRA guidelines have been followed for the preparation of the review [23]. Due to the topic of the review, a narrative review has been performed summarizing the findings.

RESULTS

The correlation between castor oil and induction

Castor oil, also known as *Oleum Palmae Christi*, is obtained from the seeds of *Ricinus communis* and has been used for centuries for its therapeutic purposes. It was first described in the Ebers Papyrus of ancient Egypt over 3,500 years ago [24]. It is a triglyceride characterized by a high content of a hydroxylated unsaturated fatty acid, the Ricinoleic acid. After the oral ingestion of castor oil, the Ricinoleic acid is released by lipases in the intestinal lumen and then absorbed, inducing a strong laxative effect [25]. The United States Food and Drug Administration classifies castor oil as a laxative, but several studies suggest its effectiveness in inducing labour [26].
Prostaglandin E2 levels in the portal vein seem to increase after the use of castor oil. Furthermore, prostaglandin E2 receptors are targets of Ricinoleic acid. The prostaglandin EP3 receptor is responsible for mediating the effects of castor oil. In fact, pharmacological and molecular biology studies have shown the presence of prostaglandin EP3 receptors in the pregnant uterus. Their activation can induce the contraction of the smooth muscle of the uterus. This molecular and physiological mechanisms explain the correlation between castor oil and labour [7, 9, 17].
The most recent guidelines indicate that labour induction with castor oil is not recommended, as the evidence does not support this method [8, 27, 28]. Nevertheless, in many centres, this "non-traditional" method of induction is routinely used. A survey among members of the American College of Nurse-Midwives revealed that 90 out of 172 midwives interviewed had used natural supplements for labour stimulation, and 93% of those who used natural methods had used castor oil [14]. In the context of out-of-hospital midwifery in the United States, castor oil is the most used method of induction in nulliparous women and the second most popular method, after membrane stripping, among multiparous women [24]. The use of castor oil as a method of labour induction was evaluated by Cochrane in 2013 with respect to a series of birth-related outcomes. The results are limited because the number of participants in the studies examined was too small to draw significant conclusions. The only result highlighted by the review is that castor oil induces nausea. In any case, the effectiveness of castor oil in inducing labour was not investigated [10].

Castor oil: induction of labour and other obstetric and neonatal outcomes

The living literature on the use of castor oil for labour induction is not extensive. The data collected from a sample of 1,653 patients indicate that the intake of castor oil increases the prevalence of vaginal births compared to the control group. Furthermore, the effectiveness of labour induction is significantly higher in the castor oil group than in the control group [29]. Administering a non-pharmacological intervention through a of castor oil promotes cervical maturation and the onset of labour [30, 31].

Regarding the association between castor oil use and the presence of meconium-stained amniotic fluid, the results are controversial. Some studies report an association between the intake of the substance and meconium-stained fluid [32], while others find no correlation in the groups studied [33, 34]. A 2022 review [35], which included 12 studies, reported data on the association between castor oil use and the presence of meconium-stained amniotic fluid in 6 of the studies. All six authors reported that there were no differences in the presence of meconium-stained amniotic fluid between the castor oil group and the control group. This information is relevant for understanding the effects of castor oil on pregnancy and childbirth, as meconium in the amniotic fluid can indicate foetal stress.

The duration of labour, including the first, second and third stage, and its total duration, seems to be shorter in the castor oil group compared to the control group [32]. However, no differences are observed concerning the outcome of "prolonged labour" when comparing castor oil to no treatment [33]. Data on the APGAR score are also conflicting. At the first minute, patients who took castor oil appear to obtain lower scores for their neonates, compared to the group without the intake of the substance [32]. However, other studies do not detect any differences between the groups examined [31, 33, 34]. Recent data from a review, indicate that there are no significant differences in the APGAR scores between the group that took castor oil and the control group [35].

The percentage of caesarean sections also seems to decrease among patients who took castor oil compared to the control group [35] and the use of castor oil does not increase the risk of caesarean section [31].

The correlation between the presence of nausea after taking castor oil has already been discussed and clarified by Cochrane [36] and other study [34, 37].

DISCUSSION AND IMPLICATIONS FOR PRACTICE

Castor oil is considered one of the so-called non-traditional methods for labour induction. It is a very ancient molecule and has been used for a long time. This concept is reinforced by literature [38], which has demonstrated that this method plays a role in increasing the rate of vaginal delivery compared to those who receive no treatment, with a high safety profile and a very low rate of adverse effects following its administration. In general, based on current literature data, it seems reasonable to consider the use of castor oil for labour induction in women with low-risk pregnancies, especially given the high rate of side effects associated with oxytocin [34, 39]. Furthermore, castor oil is a resource that can be considered among the methods for labour induction, especially in resource-poor countries where access to healthcare services is often very difficult, due to its low cost and ease of procurement.

The action of castor oil targets the receptors for prostaglandin E2, with Ricinoleic acid acting on these receptors. The EP3 receptor specifically mediates the effects of castor oil on the intestinal and uterine muscles [35]. The understanding of this mechanism of action underlies the results of other studies [40], which highlight the high rate of vaginal delivery after castor oil administration compared to control groups. Regarding the adverse effects of castor oil, the only ones described in some studies [29, 40, 41] were nausea and diarrhoea, which were never found to be debilitating for the women who took the preparation.

There are many guidelines [8, 27, 28], in the topic of labour induction available today, and in the most recent international recommendations and guidelines, the use of non-traditional induction methods, including castor oil, is not indicated. As already emphasized by a previous study, it would be useful to develop guidelines for the use of herbal medicines, particularly castor oil, in pregnant women [29]. However, considering the literature available to date, we can conclude that castor oil can still be considered, especially in countries where access to healthcare resources is difficult for most people and in low-risk pregnancies. The role of the midwife in this context is crucial, and it is therefore important for midwives to collaborate effectively with obstetricians and discuss the use of castor oil as a safe method to promote cervical dilation and prevent undue caesarean surgery [30, 42-44].

CONCLUSIONS

In this review we analysed the state of the art on castor oil, and, more generally, on the mechanisms available for the induction of labour. Castor oil, which falls under non-traditional methods for labour induction, is not recommended by major national and international guidelines. However, considering its action on the uterine muscles, its low cost, and the absence of important side effects from its administration, it is still a method that is worth to be investigated.

However, the poor methodological quality, the limited number of studies, and the heterogeneity among studies prevent a definitive assessment of the effectiveness of this method for labour induction. It's worthily to note and acknowledge among the limitation of the present review that the heterogeneity in control group selection in the cited studies, underscores the need for future studies with standardized comparison groups to provide a clearer understanding of the role of castor oil in labour induction. Future studies will help clarify which interventions are most effective and which patient populations may benefit from this approach.

Major obstetric organizations have not endorsed castor oil for labour induction. For example, the American College of Obstetricians and Gynecologists (ACOG) and the UK's National Institute for Health and Care Excellence (NICE) explicitly advise against using castor oil to induce labour, given the lack of robust evidence of benefit. These bodies prioritize induction methods with well-documented efficacy and safety profiles, a standard that castor oil does not currently meet. The above-analysed studies on castor oil are few and methodologically limited, yielding inconsistent findings. As discussed, side effects are a key concern – castor oil's cathartic action commonly causes gastrointestinal distress (nausea, vomiting, and diarrhoea). Moreover, there is apprehension about potential foetal effects; some reports observed a higher incidence of meconium-stained amniotic fluid after castor oil use, which raises concern for neonatal meconium aspiration and related complications. Considering these issues – insufficient high-quality evidence and possible risks – professional guidelines have concluded that castor oil should not be routinely used for induction.

In conclusion, high-quality evidence supporting castor oil for labour induction remains insufficient. Both ACOG and NICE emphasize that further research is needed before castor oil could be considered an evidence-based option. Any future studies would require rigorous design and larger sample sizes to conclusively determine efficacy and safety. Until such data are available, there is consensus that clinicians and patients should adhere to established medical guidelines for induction, utilizing methods with proven safety and effectiveness.

COMPLIANCE WITH ETHICAL STANDARDS

Authors' contribution

A.Me., A.Ma.: Conceptualization, writing – original draft. V.R., E.D., L.L., C.V., P.M., A.L., B.M: Writing – review & editing.

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Laparoscopic repair of caesarean scar defect (CSD): does it improve the fertility outcome?

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ABSTRACT

Objective. The rising rate of caesarean sections has been linked to potential complications that could impact subsequent pregnancies, including caesarean scar defects (CSDs). Subfertility is a common issue among women diagnosed with CSD. Various theories have been proposed to explain the connection between CSDs and secondary infertility. Several interventions have shown promise in enhancing fertility outcomes following caesarean sections, potentially reducing the reliance on assisted reproductive technology (ART) interventions. This study aimed to investigate the impact of laparoscopic repair of CSDs on fertility results.

Materials and Methods. The retrospective cohort study was conducted at the infertility and endoscopy unit at Zagazig University Hospitals between June 2020 and February 2023. All patients enrolled in the study reported subfertility and underwent transvaginal ultrasound (TVUS) with saline infusion sonography (SHG) before the laparoscopic repair procedure. Following the imaging assessments, laparoscopic repair of CSDs was performed within a few days. Subsequently, all patients were followed up for at least 24 months.

Results. In this study, twenty cases were included. Post-menstrual spotting was observed in eighteen cases (90%), while continuous bleeding occurred in two cases (10%). Among the cases, fourteen patients (70%) achieved spontaneous pregnancy within 24 months. Out of these, two patients (14.28%) experienced a miscarriage, one patient (7.14%) had an ectopic pregnancy, one patient (7.14%) delivered prematurely at 32 weeks, and ten patients (71.43%) had a term delivery.

Conclusions. Laparoscopic repair of CSDs may be a promising intervention for women with secondary infertility, leading to enhanced chances of achieving pregnancy.

INTRODUCTION

Since the last decade, the rate of caesarean sections (CS) has continued to grow worldwide. In the Netherlands, the CS rate increased from 7.4 to 15.8% during the period from 1990-2008, and in the U.S.A., it increased from 21.2 to 32.8% from 1990 to 2011 [1]. Disappointingly, Egypt occupies the top of Africa in CS deliveries, as it represents approximately 51.8% of the year's deliveries [2].

Additionally, Emergency primary CS is associated with a higher incidence of CS scar defects, with less healing process [3].
The increased CS rate is associated with certain complications that may affect future pregnancies, such as placental abnormalities, uterine rupture, and CS scar defects (CSDs) [4]. Moreover, the relationship between subfertility and CS has been recorded, where subfertility may be a consequence of CS [5].
A recent meta-analysis including 750,407 women reported an increased time to conceive and risk of subfertility among women with a history of caesarean delivery when compared to women who delivered vaginally [6].
Recent studies have indicated that subfertility is a prominent symptom in women diagnosed with CSD [7]. The appearance of CSDs has attracted great attention in the last two decades [8].
The term CSDs is used to describe all abnormalities characterized by a defect within the myometrium (more than 2 mm) at the site of a previous CS, as mentioned in modified Delphi criteria using vaginal ultrasound, a pouch at the anterior uterine wall, or adhesions at the site of a CS incision by hysteroscopic examination due to defective healing [9].
Several hypotheses have tried to clarify the association between CSDs and secondary infertility [10], including detrimental environments for sperm penetration and implantation (such as the presence of intrauterine fluid, blood, and mucous, which can affect embryo implantation physically, and/or abnormal uterine contractility) [11].
Various interventions have been developed aiming to improve the gynaecological symptoms of CSDs, including hysteroscopic niche resection (HNR) [12], vaginal niche resection (VNR), laparoscopic niche resection (LSNR), and laparotomic niche resection (LTNR) [13]. The Treatment plan should be individualized depending on the presenting symptoms, like secondary infertility, the size of the defect, and future conception plans. The small defects can be repaired by hysteroscopy, and larger defects are managed by laparoscopy, vaginal approach, and combined hysteroscopy and laparoscopy [14].
However, the effect of these interventions on fertility outcomes remains unclear [15, 16].
Laparoscopic repair of caesarean scar defects (CSD) offers several unique advantages over traditional surgical methods: Symptom Improvement: Approximately 77% of patients experienced relief from symptoms such as abnormal uterine bleeding and pelvic pain following laparoscopic niche repair and Fertility Restoration. The procedure restored fertility in about 73% of patients, with a reduced time to conception post-surgery [17].
This may be achieved by Anatomical Restoration as Laparoscopic repair effectively restores the thickness of the myometrium, enhancing uterine integrity.
Comprehensive Pelvic Assessment thorough exploration of the pelvic cavity, enabling the identification and treatment of additional pathologies [18].
Moreover, laparoscopic repair without scar resection is considered a feasible, safe, and straightforward approach to treating CSD [19].
It was hypothesized that all interventions used to treat CSD can help in improving the fertility outcomes after CS and may decrease the burden of assisted reproductive technology (ART) needs [13].
Unfortunately, the evidence needed to justify the implementation of these surgical interventions on the reproductive outcome is still deficient [20].
Thus, this study was designed to provide an overview of the effect of laparoscopic repair of CSDs on fertility outcomes in women suffering from delayed conception.

MATERIALS AND METHODS

This was a retrospective cohort study conducted at the endoscopy and infertility unit of Zagazig University Hospitals between June 2020 and February 2023.

Patients

Data from all women enrolled in laparoscopic repair of CSDs reporting subfertility (failure to conceive for at least 12 months) with at least one of the following (post/intermenstrual spotting, pelvic pain, or dyspareunia) were included in this study. Women who had a previous hysterotomy (less than 28 weeks), a history of placenta previa, and/or morbidly adherent placenta in a previous CS delivery, patients with hydrosalpinx, poor ovarian reserve and those with male factors of infertility were excluded from the study.

Ultrasonic review

Transvaginal ultrasound (TVUS) with saline infusion sonography (SHG) (**Figure 1**) was scheduled before laparoscopy, and the CSD was measured using modified Delphi criteria. CSD is formally defined as a ≥ 2 mm indentation of the uterine myometrium at the site of the caesarean scar assessed by transvaginal ultrasound. Moreover, the shape of

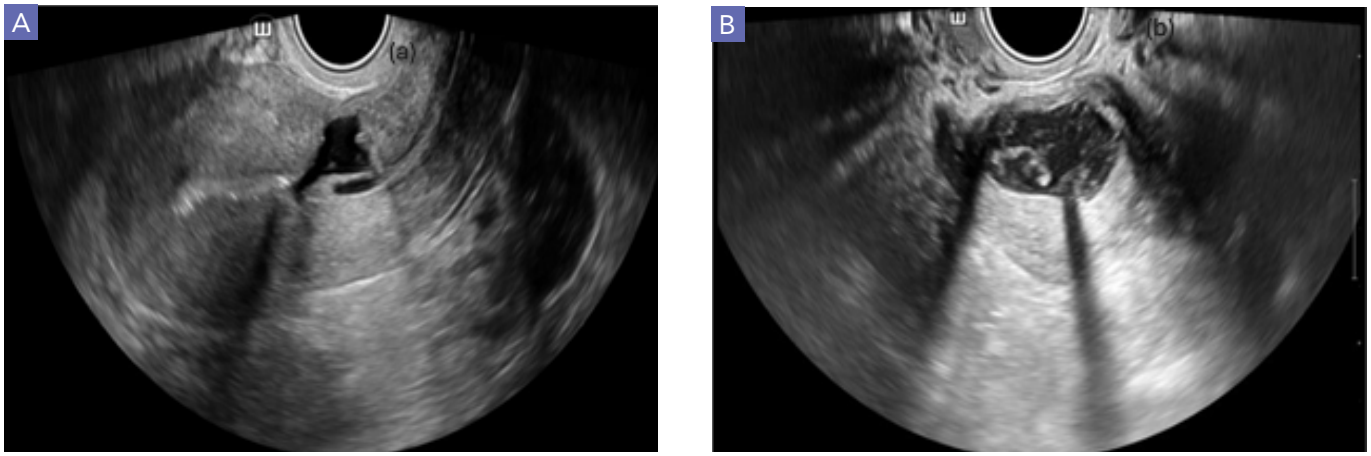

Figure 1. *Sonographic planes needed for caesarean scar niche measurement.*
(A) The sagittal plane; **(B)** The transverse plane.

the CSD was registered as triangular, semicircular, rectangular, circular, droplet, or inclusion cysts [8].

Laparoscopic repair

Laparoscopic repair of CSDs was conducted a few days later; primary laparoscopic entry was done through the Lee Huang point, followed by three secondary ports (two lateral ports and one suprapubic). Mobilization of the bladder was performed, then cervical dilatation was done vaginally up to Haegar No. 10. The CSD was identified using intentional sound perforation. Opening the assumed CS scar and trimming the edges were performed to obtain healthy bleeding edges. Finally, the defect was sealed using a 0/0 Vicryl suture in an interrupted single-layer manner. (**Figure 2**).

Pregnancy follow-up and outcomes

Sexual abstinence was advised for the 3 months following the surgery. Thereafter, normal sexual life was regained. All patients were followed up for a maximum of 24 months.

Statistical analysis

Statistical analysis was done by SPSS v26 (IBM Inc., Armonk, NY, USA). The Shapiro-Wilks test and histograms were used to evaluate the normality of the distribution of the data. Quantitative parametric data were presented as mean and standard deviation (SD). Quantitative non-parametric data were presented as the median and interquartile range (IQR). Qualitative variables were presented as frequency and percentage (%). A paired sample t-test was used to compare the population means of two correlated samples. A two-tailed P-value < 0.05 was considered statistically significant.

RESULTS

Twenty cases were enrolled in this study. The mean age was 27.6 ± 3.89 years, while the mean BMI was 25.5 ± 3.4 kg/m^2. Thirteen (65%) patients had one previous CS, 6 (30%) had two previous CS, and 1

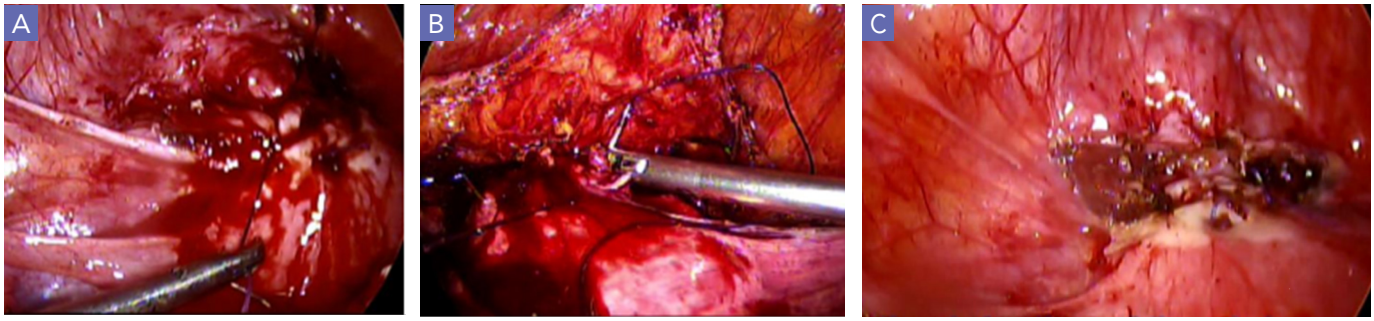

Figure 2. *(**A, B**) Suturing the edges using 2/0 polygalactin 910 sutures in an interrupted manner using the intracorporeal suture technique. We used a single-layer technique and didn't close the visceral peritoneum; (**C**) Final view of the repair.*
Number of sutures according to the size of the niche, ranging 3-5.

Table 1. *Demographic data regarding the fertility.*

	n = 20
Age (years)	27.6 ± 3.89
BMI (kg/m²)	25.5 ± 3.4
Number of CS	
One	13 (65%)
Two	6 (30%)
Three	1 (5%)
Time since last CS (months)	30.8 ± 10.4

Data are presented as mean ± SD or frequency (%); BMI: body mass index; CS: caesarean section.

Table 2. *Bleeding pattern and position of uterus.*

		n = 20
Bleeding pattern	Post menstrual spotting	18 (90%)
	Continuous	2 (10%)
Position of uterus	AVF	16 (80%)
	RVF	4 (20%)

Data are presented as frequency (%); AVF: anteverted anteflexed, RVF: retroverted retroflexed.

Table 3. *Intraoperative data.*

	n = 20
Operative time (min)	86.7 ± 20.6
Tube	
Normal	18 (90%)
Unilateral block	2 (10%)
Adhesion	
Massive	2 (10%)
Mild	8 (40%)
No	10 (50%)

Data are presented as frequency (%).

Table 4. *Menstrual characteristics and visual analogue scale before and after CSD repair.*

	Baseline	After	P-value
Menstrual characteristics			
Number of days with spotting	6 (4.75-7.25)	0 (0-0)	< 0.001*
Dysmenorrhoea (VAS)	2 (1-2)	1 (1-1)	0.006*
Dyspareunia (VAS)	3 (2-3)	1 (0-1)	< 0.001*
Chronic pelvic pain (VAS)	2 (1.75-2)	0 (0-1)	< 0.001*

Data are presented as median (IQR); VAS: visual analogue scale; *statistically significant as P-value < 0.05.

(5%) had three previous CS. The mean time since the last CS was 30.8 ± 10.4 months. No medical or surgical comorbidities could be reported (**Table 1**).

Eighteen (90%) patients had post-menstrual spotting while 2 (10%) had continuous bleeding. The position of the uterus was AVF in 16 (80%) patients, while RVF was found in 4 (20%) patients (**Table 2**). The tubal patency test revealed that 18 (90%) patients were found to have normal tubes, whereas 2 (10%) patients had unilateral blocks. Regarding pelvic adhesion, 8 (40%) patients had mild adhesion, while 2 (10%) of them had massive adhesion. The mean operative time was 86.7 ± 20.6 min (**Table 3**). The days of post-menstrual spotting, dysmenorrhea, dyspareunia, and chronic pelvic pain using (VAS) were significantly reduced after repair compared to baseline ($p < 0.05$) (**Table 4**).

Regarding fertility, 14 (70%) patients got pregnant spontaneously within 24 months 2 patients (14.28%) had a miscarriage, 1 (7.14%) patient had an ectopic pregnancy, 1 (7.14%) patient had preterm delivery at 32 weeks, and 10 (71.43%) patients had term delivery (**Tables 5, 6**).

DISCUSSION

A global increase in the rate of CSs has led to a greater need for awareness regarding the complications that can arise following a CS [21]. CSDs are recognized as long-term complications that can arise following CS [22]. The association between CS and secondary infertility is hypothesized to be mediated by the presence of CSDs [10].

There have been numerous interventions developed and widely used to treat the gynaecological symptoms associated with CSDs. These interventions have been suggested as potential alternatives or additions to ARTs. However, there is a lack of evidence supporting the use of these surgical interventions for reproductive purposes [23].

This study aimed to follow up on the reproductive outcomes after laparoscopic CSD repair.

In patients suffering from secondary infertility. Our results confirmed that 70% of patients regained their spontaneous ability to conceive within 24 months following laparoscopic repair. Out of them, 71.4% had term delivery. In addition, 7.1% of patients had preterm delivery at 32 weeks, 14.2% of patients had miscarriage and 7.1% of patients had an ectopic pregnancy.

In a recent study including 15 patients desiring fertility, 11 patients (73%) got pregnant after laparoscopic CSD repair with 9 patients (60%) having live birth while there were two miscarriages. Nine

Table 5. *Niche characteristics of the patients studied before and after CSD repair.*

	Baseline	After	P-value
Length (mm)	12.29 ± 4.03	5.08 ± 1.48	< 0.001*
Depth (mm)	6.79 ± 1.71	2.95 ± 0.83	< 0.001*
Width (mm)	15.55 ± 4.9	7.95 ± 1.99	< 0.001*
Shape			0.015*
Triangular	9 (45%)	15 (75%)	
Semicircular	1 (5%)	5 (25%)	
Rectangular	5 (25%)	0 (0%)	
Isthmocele	2 (10%)	0 (0%)	
W-shape	2 (10%)	0 (0%)	
Droplet	1 (5%)	0 (0%)	
RMT (mm)	2.38 ± 1.12	6.88 ± 1.46	< 0.001*
AMT (mm)	11.4 ± 2.9	11.78 ± 1.88	0.638

Data are presented as mean ± SD or frequency (%); RMT: residual myometrial thickness; AMT: adjacent myometrial thickness; *statistically significant as P-value < 0.05.

Table 6. *Pregnancy outcome after CSD repair.*

	n = 14
Pregnancy outcome	
Aborted	2 (14.28%)
Preterm 32w	1 (7.14%)
Term	10 (71.43%)
Tubal ectopic	1 (7.14%)

Data are presented as frequency (%).

conceived spontaneously and two conceived via ART. No serious complications were reported, including caesarean scar pregnancy or uterine rupture, following the laparoscopic repair [17].

Additionally, Tanimura *et al*. mentioned comparable results as they found that 10 out of 18 (55.6%) patients who underwent laparoscopic CSD repair could achieve pregnancy within 9 months as a medium period. Five patients delivered at term, 3 had a preterm delivery, and 2 had a miscarriage [24].

Lv *et al*. stated that 8 (61.5%) out of 13 patients who wished to conceive were able to achieve pregnancy; 2 had term delivery, 3 had preterm delivery and 3 had a miscarriage [25].

Moreover, Karampelas *et al*. reported that out of 31 patients who underwent laparoscopic repair of the CSD, 10 out of 12 people with secondary infertility conceived spontaneously following surgery. These findings support the high incidence of fertility restoration after laparoscopic CSD excision. Only one patient had placenta previa, and none of them had uterine dehiscence or rupture [26].

Additionally, in a case series done to evaluate the fertility outcomes after laparoscopic CSD repair including 18 women with infertility, 8 (44%) patients became pregnant and delivered healthy babies by caesarean section at 38-39 weeks of gestation [27].

In contrast to earlier findings, Vervoort *et al*. studied 101 cases complaining of CSD-related symptoms, and they reported only 2 cases getting pregnant with no other data in their study regarding infertility and pregnancy outcomes [12]. Wu *et al*., who used combined laparoscopy and hysteroscopy techniques, found that only one case out of 25 cases got pregnant after CSD repair despite a 1-year follow-up [28].

Unfortunately, all the trials were not RCT. However, Vissers *et al*. submitted their LAPRES trial protocol investigating the effects of laparoscopic repair of CSD on reproductive outcomes in an RCT manner under registry no. NL6350. This is an ongoing study, and the results have not yet been published [29].

It is of note that in most of the studies examining the impact of CS scar repair on fertility outcomes, hysteroscopic repair was the preferred method. This is due to being a minimally invasive procedure, shorter hospital stays, and the familiarity among surgeons, as opposed to laparoscopy which requires a certain level of expertise.

Gubbin *et al*., in 2011, reported comparable results as they mentioned that all patients regain their ability to conceive spontaneously within 24 months after hysteroscopic repair of CSD, but they did not mention their pregnancy outcome [30].

Another study done on a small number of patients confirmed similar results as they observed that spontaneous pregnancy was seen in 10 out of 12 (83.3%) participants presenting initially with secondary infertility [26].

Moreover, in an RCT, done to compare the effect of hysteroscopic repair and expectant management on fertility outcomes, they found that pregnancy was significantly higher in the repair group (75%) when compared to the expectant management group (32.1%, p = 0.001). Among the cases who got pregnant in the repair group, 19.05% had spontaneous miscarriages, and (80.95%) were delivered by caesarean section. Rupture of uterine scars did not occur in any of those treated patients [31]. Additionally, Cohen *et al.* in 2020 confirmed that hysteroscopic resection of CSD may improve fertility outcomes as 58.4% of their patients had conceived spontaneously as well as 13.2% after IVF [32].

When comparing the fertility outcomes following different methods of intervention (hysteroscopic, vaginal, and laparotomy repair), Vissers *et al*. reported that 11 out of 12 (92%) patients got pregnant. There was no significant difference between the three surgical procedures regarding the time to conception (14 months on average), type of conception (spontaneous or by ART), or mode of delivery [33]. Nezhat *et al*. observed that the laparoscopic repair of caesarean scar defects (CSD) resulted in significant symptom improvement in 77% of patients and restored fertility in 73% of patients [17].

Comparatively, other studies have reported varying outcomes:

- A novel laparoscopic technique without scar resection demonstrated a significant increase in residual myometrial thickness and alleviation of postmenstrual bleeding, suggesting its effectiveness in treating CSD [19].
- Single-incision laparoscopic repair is a feasible approach, offering excellent cosmetic results and potentially reducing surgical invasiveness [34].

These comparisons indicate that our outcomes are consistent with existing literature, supporting the efficacy of laparoscopic CSD repair in symptom relief and fertility restoration.

Our findings show a significant reduction in all caesarean scar (CS) niche dimensions (length, width, and depth) after the procedure, along with a remarkable increase in residual myometrial thickness (RMT) from 2.38 ± 1.12 mm to 6.88 ± 1.46 mm ($p < 0.001$). This improvement is more than just a number, it reflects better healing of the uterine wall, which is crucial for women planning future pregnancies. A thinner or weakened lower uterine segment, often seen after a caesarean section, due to reduced RMT and persistent CS defects are a major risk factor for abnormal placentation (*e.g*., placenta accreta spectrum disorders) [35]. This improvement in uterine integrity may play a crucial role in enhancing post-CS uterine healing, reducing the likelihood of abnormally invasive placentation in future pregnancies, potentially lowering the risk of severe obstetric complications, including life-threatening haemorrhage and hysterectomy.

By restoring uterine integrity, this procedure may reduce the chances of these life-threatening complications, providing not only symptom relief, but also a safer environment for future pregnancies. This highlights the importance of proactively addressing CS scar defects, not just for immediate symptom control, but for long-term reproductive health.

Limitations

While this study provides valuable insights into the impact of laparoscopic repair of CSDs on fertility outcomes, several limitations should be acknowledged:

- Small Sample Size: the study included only 20 patients, which limits the generalizability of the findings. Larger, multi-centre studies are needed to confirm these results.
- Retrospective Study Design: as a retrospective cohort study, the analysis is subject to potential selection bias and recall bias, which may affect the accuracy of reported outcomes.
- Lack of a Comparative Control Group: the absence of a control group (*e.g*., patients who did not undergo laparoscopic CSD repair) makes it difficult to determine whether the observed improvements in fertility outcomes were directly attributable to the intervention.
- Limited Follow-Up Data**:** although patients were followed for a maximum of 24 months, long-term data on subsequent deliveries, uterine health, and potential complications (*e.g*., uterine rupture, placenta previa) remain unavailable.

Future prospective studies with larger cohorts, longer follow-up durations, and comparative control groups are recommended to further evaluate the effectiveness and safety of laparoscopic CSD repair in improving fertility outcomes.

CONCLUSIONS

In conclusion, it is possible to hypothesize that the repair of CSD is followed by an increased pregnancy rate regardless of the method of repair and most patients can regain their spontaneous ability to conceive within 18-24 months following CSD repair.

In addition, patients who had failed ART trials before CSD repair also appeared to have a better outcome after repair. The pregnancy outcomes and mode of delivery after CSD repair look unaffected by the method of repair used in different studies. Uterine rupture appeared to be an uncommon complication following CSD repair regardless of the method of intervention.

Regarding CSD repair, particularly in patients with secondary infertility, laparoscopic repair may be a good option as it provides additional benefits for infertility management such as adhesiolysis and testing tubal patency.

COMPLIANCE WITH ETHICAL STANDARDS

Authors' contribution
M.I.K., D.O.E.: Conceptualization, resources. A.A.A., M.A.I.: Methodology, data curation, software. M.A.H., A.A.A.: Investigation. M.A.I., M.I.K.: Validation, formal analysis. D.O.E., M.A.H.: Supervision, project administration. M.A.H., S.A.S.: Writing – original draft, visualization, Writing –review & editing.

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Study registration
N/A.

Disclosure of interests
The authors declare that they have no conflict of interests.

Ethical approval
The study was approved by the Institutional Research Review Board of Zagazig Faculty of Medicine (IRB) under reference number (ZU- IRB# 384/26-May-2024) and by Zagazig Hospitals Administration. All experiments were performed by relevant guidelines and regulations and participants were not exposed to any harm or unintended effect. The study followed the ethical principles of the Declaration of Helsinki.

Informed consent
Informed consent to participate in the study was taken from all the participants after explaining the study objectives, and measures, and ensuring confidentiality.

Data sharing
Data are available under reasonable request to the corresponding author.

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